IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of

Anderson Confirmation No. 7327

Serial No. 09/994,937 Group Art Unit 1616

Filed November 28, 2001 Examiner Webman

For SOLVENT SYSTEMS FOR PHARMACEUTICAL AGENTS

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF DAVID M. ANDERSON
UNDER 37 C.F.R. §1.132

David M. Anderson declares as follows:

1. I am the inventor of the above-identified application. I hold a position in Lyotropic Therapeutics, Inc., the assignee of record of the above-identified application. I have read and understand the application, and I have read and understand the office action mailed April 6, 2006. I have also read and understand the references of record.

2. I am an expert in the fields of chemical formulations and drug delivery, particularly as applied to structured fluids including emulsions, liposomes, lyotropic liquid crystals, including reversed cubic and reversed hexagonal phase materials, and the like. As evidence of my expertise, I have attached hereto my curriculum vitae (CV) as **Attachment 4**. I hold the degree of Masters in Mathematics and Ph.D. in Chemical Engineering. I have authored over twenty papers which appear in refereed journals, and I am a highly skilled investigator competent to conduct experiments on structured fluids and to utilize equipment for properly characterizing the nature of such fluids. Based on my education, training and experience as set forth in the attached CV, I am qualified to provide opinion evidence on the level of skill of one of ordinary skill in the art, and as to what would be obvious or not obvious to one of ordinary skill in the art. In addition, I am qualified to conduct experiments and to provide test results relating

to various chemical formulations.

3. I have conducted experiments that, when interpreted in light of the established literature in the field show that:

(i) as is well known in the art, relatively small variations in the relative concentrations of the same ingredients can give widely and radically different results in terms of the underlying structure; thus, in the particular case of combining a representative mixture taught by U.S. Patent 6,458,373 to Lambert with spearmint as taught by U.S. Patent 5,716,928 to Benet and/or gentisic acid as taught by U.S. Patent 4,440,739 to Azuma, a change from liquid crystalline phase to liquid phase was observed over a relatively small concentration range; and

(ii) no combination of the representative Lambert mixture with spearmint oil and/or gentisic acid produces, or are capable of producing, a reversed cubic or reversed hexagonal phase compositions as claimed in the instant application.

These experiments flatly disprove the conclusion that systems described in Lambert, allowing for the addition of spearmint oil and/or gentisic acid, can yield the reversed cubic liquid crystalline phases or reversed hexagonal liquid crystalline phases contemplated in the claims of my U.S. Patent Application 09/994,937. Therefore, they disprove the assertion that the compositions claimed by the instant application are obvious in light of Lambert, Benet and Azuma.

(a) Experimental Approach: First, an exhaustive phase behavior study was performed to delineate the phases present in mixtures of water, spearmint oil, and the mixture (herein referred to as "Mixture A") of ascorbyl palmitate (triethanolamine salt), vitamin E, and TPGS (tocopheryl polyethyleneglycol 1000 succinate) used in representative Example 4 of the Lambert patent in which the anticancer drug paclitaxel was solubilized.

Second, these results were interpreted against the background of five decades of published surfactant phase behavior work, which unequivocally shows that reversed liquid crystalline phases cannot be present in the Lambert system at any composition of Mixture A, water and spearmint.

Third, to various samples were created by combining Mixture A and Spearmint, gentisic acid was added, as the Examiner states was taught by Azuma, and the phases present in these mixtures were identified and compared to those of the samples without gentisic acid.

(b) Experimental Procedure: Vitamin E TPGS (tocopheryl polyethyleneglycol 1000 succinate), in the amount 15.03 grams, was dissolved in methanol along with 1.877 gm ascorbyl palmitate, 0.56 gram triethanolamine, and 22.49 gm alpha-tocopherol (vitamin E). This mixture was then put on a rotovap to evaporate the methanol, leaving Mixture A. Note that as in Lambert, the addition of triethanolamine converts the ascorbyl palmitate to the salt form.

This Mixture A was then combined with water and with essential oil of spearmint ("spearmint oil") at 46 (forty six) different ratios which cover the essential portion of the pseudo-ternary phase diagram, sufficient to locate any liquid crystalline phases present. The phase diagram was mapped at room temperature.

Each of the samples was thoroughly mixed, centrifuged, photographed, and then examined with polarizing microscopy. A Reichert-Jung Polyvar microscope with 10x, 25x, 40x, and 100x objectives was used with crossed polarizers inserted, with most of the work being performed with the 40x oil immersion objective; eyepiece magnification provided another 6.3x factor. Particular note was made also of the viscosities of the phases present. Phases of the various samples were identified and recorded, and a phase diagram prepared.

Thereafter, in order to test experimentally whether the addition of gentisic acid to the Lambert system could induce changes in phase behavior, in particular introduce new reversed liquid crystalline phases, an amount of gentisic acid equal to 10% by weight of the sample was added to the 10 samples numbered A0 through A9 and the mixtures equilibrated. Phases of the various samples were identified and recorded.

(c) Phase behavior results: **Table I**, attached to the end of this Declaration, gives the compositions of the 46 samples, the observed phases, and the microscopy observations. Photographs of the test tubes, and optical micrographs supporting the phase determinations, are available.

Based on these results, the approximated pseudo-ternary phase diagram was assembled, and is shown in **Figure 1**, which is attached to the end of this Declaration.

Table II, attached to the end of this Declaration, gives the compositions of the 10 samples of Mixture A and amounts of gentisic acid with which they were

combined, the observed phases, and the microscopy observations. After equilibration, the presence of crystals of gentisic acid in polarizing optical microscopy examination of the ten samples demonstrated that sufficient gentisic acid had been added, since the crystals represent the excess gentisic acid over what is solubilized. These crystals are clearly visible in the photomicrograph which is **Figure 2** attached to the end of this Declaration. Since the amount of crystalline material present can be visibly seen to be far less than 10%, this means that a significant portion of the gentisic acid has dissolved. However, *none of the samples A0 through A9 changed phase* upon addition of gentisic acid.
US Pat App Ser No. 09/994,937 (at Paragraph 0255, page 48 at line 7 et seq.) contains a section explicitly devoted to the effect of gentisic acid on phase behavior of surfactant systems, and states "..when a phosphatidylcholine-water mixture is converted to a non-lamellar phase by the addition of an essential oil or other compound, then the addition of gentisic acid salts such as gentisic acid ethanolamine, and related compounds as described herein, have a tendency to cause the mixture to revert back to lamellar...". Thus, in direct experimentation that is reported in the instant application, the inventor, an expert in the phase behavior of surfactant systems, reports that gentisic acid actually causes the *exact opposite* of inducing reversed liquid crystalline phases. Gentisic acid was added in an amount greater than would be used in a typical antioxidant in a pharmaceutical formulation, and still no changes in phase and no reversed cubic or reversed hexagonal phase material were observed. The addition of lesser amounts of gentisic acid would have even less impact on creating reversed cubic or reversed hexagonal phase materials.

(d) Region Assignments: Because the number of components far exceeds that of a true ternary phase diagram (3), there is more overlap between the various phases than are suggested by a literal interpretation of the diagram; thus, for example, excess oil tends to be present in many of the samples. Also, the micellar phase in Region 4 is technically a microemulsion phase since it contains oil. And the high oil contents present in most samples meant that emulsions inevitably formed particularly at the higher water contents; while an emulsion is not an equilibrium phase, one can deduce the equilibrium phases present in this system even at high water content when emulsions are present, because one can

extrapolate from the phase behavior at lower water contents.

In spite of the complexity brought about by the introduction of the spearmint oil, this phase behavior work demonstrates quite clearly that the cubic phase of Region 3 lies at a higher water content than the lamellar phase of Region 2. As discussed herein, this phase progression, in the present system comprising only water-soluble surfactants, leads directly to the assignment of the cubic phase as a normal (Type I) cubic phase, and precludes the possibility of reversed (Type II) liquid crystalline phases appearing at higher water content.

A series of micrographs set forth in **Attachment 1** reveals the liquid crystalline structures visible in polarizing microscopy. The first micrograph reveals the lamellar liquid crystalline textures of sample B1. The next micrograph shows the same sample viewed macroscopically through crossed polarizers, making the birefringence from the lamellar phase plainly visible as a pronounced shining. The third micrograph below then shows the isotropic nature of sample B2 at higher water content. Note the absence of birefringent optical textures, in contrast with the micrograph above.

As discussed in exhaustive detail below, the assignment of the phases present in this phase diagram is based on a combination of polarizing optical microscopy, viscosities, and a wealth of consistent information from the published work on lyotropic phases. Most importantly, the progression of lamellar to cubic as the water content is increased, combined with the high HLBs of the surfactants involved, unequivocally proves that the cubic phase is of the normal type (or "Type I"), not the reversed type ("Type II") now specifically claimed in U.S. Patent Application Serial No. 09/994,934. This conclusion is supported overwhelmingly by many years of published phase behavior work discussed below.

I have provided some definitions in **Attachment 2** for better understanding the explanation below. These definitions are completely consistent with the definitions of terms given in a number of patent disclosures by Anderson, including the instant US Patent Application 09/994,937, with the most comprehensive in terms of definitions being U.S. Patent No. 6,482,517. Furthermore, they are universally consistent with the large volume of published literature from a wide range of authors that is cited or referred to below.

A universal principle recognized by those skilled in the art and found in the literature is that whenever a lamellar phase containing water, optionally oil, and one or more water-soluble surfactants all of high HLB is convertible to a cubic or hexagonal phase by the addition of water, then the resulting cubic or hexagonal phase is of the normal (Type I) variety. The counterpart to this Principle is also universally true, namely that whenever a lamellar phase containing water, optionally oil, and one or more water-insoluble surfactants all of low HLB is convertible to a cubic or hexagonal phase by the reduction of water content, then the resulting cubic or hexagonal phase is of the reversed (Type II) variety. This rule is supported by vast research in lyotropic liquid crystals. I will refer to these two principles collectively as the "Principle". The phase behavior pattern embodied in this Principle is central to the many well recognized publications in the field, as are set forth in **Attachment 3**, several of which are held in the highest regard in the history and understanding of lyotropic liquid crystals.

As an expert in the field, it is my opinion that there is no known exception to this rule. I am the author of 20 peer-reviewed publications in the field of lyotropic liquid and liquid crystalline phases, and have mapped out many phase diagrams and on a regular basis I must make determinations of lyotropic phases as part of my research and development work for my employer Lyotropic Therapeutics, Inc. Such determinations have always been in accordance with this Principle.

In my opinion, application of this Principle in the present case means that the systems in Lambert cannot, even with the addition of spearmint oil, yield the reversed cubic and reversed hexagonal liquid crystalline phases now claimed in U.S. Patent Application Serial No. 09/994,934. Nor can the systems in Lambert in combination with Benet, even with the further addition of gentisic acid as taught in Azuma, yield the reversed cubic and reversed hexagonal liquid crystalline phases now so claimed.

It is widely known that tocopheryl polyethylene glycol ("TPGS") is a water-soluble surfactant of high HLB. For example, U.S. patent no. 6,241,969 to Saidi states that "...TPGS has an HLB between about 15 and 19." A salt of ascorbyl palmitate, which has a polar group of MW equal to 203 plus the weight of the counterion—150 in the case of Lambert, for protonated triethanolamine,

making a total head group MW of 353—and a hydrophobic group of only 211 is clearly a high-HLB surfactant (greater than 10), and this assignment is supported by the fact that it is highly water-soluble.

Therefore, this Principle demonstrates that the cubic phase in the above phase diagram is in fact of the normal, Type I variety. There are no reversed (Type II) liquid crystals in the Lambert system, not even at water contents far lower than the excess water present in the emulsion systems of Lambert. Nor do reversed cubic or reversed hexagonal liquid crystalline phases appear when spearmint oil is added to the Lambert system, again over a range of water contents much larger than that of the Lambert disclosure.

(e) Conclusions: Direct experimentation and analysis shows that the compositions used in Lambert for the solubilization of paclitaxel do not and, even with the addition of spearmint oil as taught by Benet and gentisic acid as taught by Azuma, cannot produce the reversed (Type II) cubic or hexagonal liquid crystalline phases of U.S. Patent Application Serial No. 09/994,937.

Although the various combinations are made of identical ingredients, because their relative composition is changed they create fundamentally different morphologies and thus compositions from one another. Furthermore, at the high water contents that characterize the emulsions of focus in both the text and claims of Lambert, any liquid crystalline phases present could only be those (for example normal hexagonal, normal cubic) that are on the opposite end of the spectrum of liquid crystal morphologies from the reversed cubic and reversed hexagonal phases of U.S. Patent Application Serial No. 09/994,937. They would be incapable of solubilizing a compound, such as a drug, which is "otherwise les than 5% by weight soluble in soybean oil" as set forth in the claims of U.S. Patent Application Serial No. 09/994,937, in a vehicle that is dispersable in water.

The importance of reversed (Type II) cubic and hexagonal phases over their normal (Type I) counterparts in the invention claimed in U.S. Patent Application Serial No. 09/994,937 is in part a direct reflection of the relationship between insolubility and Type. That is, the insoluble Type II phases do not readily dissolve in relevant aqueous fluids such as blood, GI fluids, lymph, and cytoplasm but rather tend to retain their integrity in these fluids, which is obviously crucial in applications such as drug-delivery.

4. In my opinion, one of ordinary skill in the art of lipid based drug delivery would typically have received education leading to the degree of Ph.D. in Chemical Engineering, Chemistry, Biochemistry or a related field. He or she would have likely engaged in research at the Post Doctoral level, and may have been the lead author on a few articles in refereed journals. He or she would typically have had 5-10 years experience in research and development, and would be familiar with and adept at conducting the type of experiments set forth in item 3 above, and would be adept at preparing phase diagrams as shown in Figure 1 above. He or she would recognize and understand the differences between emulsions, liposomes, liquid crystalline phase materials (and would recognize differences between cubic phase liquid crystalline phase materials and reversed cubic liquid crystalline phase materials, and hexagonal liquid crystalline phase materials and reverse hexagonal liquid crystalline phase materials), and would know how test for the presence of physically different attributes. In my opinion, **Table III** which I have prepared, is a concise summary of some of the major differences among reversed cubic and hexagonal phase lyotropic liquid crystalline material and emulsions, as known to those skilled in the art. **Figure 3** are TEM photomicrographs of reversed cubic and reversed hexagonal phase lyotropic liquid crystalline material and an emulsion.

In my opinion, one of ordinary skill in the art would not seek to combine teachings of the Lambert, Benet or Azuma references as there is no teaching, suggestion or motivation to do so in any of the three references. The three references are drawn to different subject matter and solve different problems. Specifically, Lambert describes an emulsion vehicle for poorly soluble drugs, Benet describes using essential oils to increase the bioavailability of oral pharmaceutical compounds without reference to solubilization but instead specifically explained with reference to intracellular efflux inhibition systems, and Azuma teaches a radioactive diagnostic agent (as opposed to a pharmaceutical) and mentions various classes of additives in passing.

In my opinion, one of ordinary skill in the art would be aware that physical differences between compositions made of the same or similar compounds can have profound effects on the use and application of those compositions. Further,

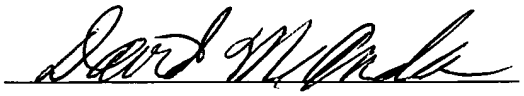
in my opinion, one of ordinary skill in the art would recognize that varying the ratios of constituents may have profound effects on the physical entity which is achieved. That is, one of ordinary skill in the art would be aware that simply mixing ingredients together may not result in obtaining a specific physical entity, other factors such as the relative amounts of the ingredients may need adjustment, and such adjustment would not be performed unless a specific physical entity was desired (i.e., without a motivation to make a liquid crystalline phase material, one of ordinary skill in the art would not seek to do so). Finally, in my opinion, one of ordinary skill in the art would recognize that not every collection of constituents can be combined to make certain physical entities. For example, as noted above in item 3, the materials of Lambert, Azuma and Benet cannot be combined to make reverse cubic liquid crystalline phase materials or reverse hexagonal liquid crystalline phase materials.

Therefore, it is my opinion, based on the experimental results noted above in item 3 (which one of ordinary skill in the art would be able to duplicate), based on the level of skill of those of ordinary skill in the art, and based on the divergent subject matter described in Lambert, Benet and Azuma, one ordinary skill in the art would be aware that simply mixing and matching chemicals does not necessarily yield a reversed cubic liquid crystalline phase or reversed hexagonal liquid crystalline phase and that with particular reference to a combination of Lambert, Benet and Azuma, would not yield a reversed cubic liquid crystalline phase or reversed hexagonal liquid crystalline phase.

5. As a means of further explaining the information embodied in a phase diagram and its central role in identifying and differentiating phase behavior in lyotropic liquid and liquid crystalline systems, I reference The Colloidal Domain, 2nd edition, D. Fennell Evans and Hakan Wennerstrom, Wiley-VCH, New York, 1999 at section 10.1 (pages 493-538).Chapter 10, Phase Equilibria, Phase Diagrams and their Application. On Pages 502-504 the author explains the construction of a phase diagram (Figures 10.6 and 10.7) with reference to a ternary system water – potassium decanoate (caprate) – octanol, at constant pressure and temperature. Each corner represents a pure component, while the opposite base of the triangle represents zero of that component, hence a binary system of the other two

components. We arrive at the relative amount of component A at any point P by drawing a line parallel to the A base. Every unique point within the triangle represents a unique ration of the three components. Varying ratios of the three compositions create regions of very different morphologies or phases, including in this particular case lamellar, normal hexagonal, reversed hexagonal, micellar and reversed micellar. From Figure 10.6 it can be seen that at very high concentration of octanol, and equal concentrations of water and potassium caprate, a reversed micellar phase is created (at the top of the triangle). By substantially lowering the concentration of octanol in the composition, but maintaining equal concentrations of water and potassium caprate, a normal hexagonal phase morphology material is created. Increasing the concentration of water yields a normal micellar phase. It was following this same technique that I assigned compositions to the phase diagram set forth in Figure 1 above, and interpret the results.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Date: 12-12-06

David M. Anderson

Table I

Mixture A of Lambert with varying amounts of Spearmint

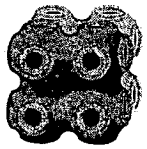
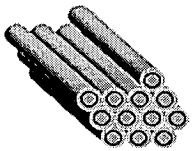
ID	Mix A (%)	Spear (%)	Water (%)	Description of phases present
A0	100	0	0	Bottom phase lamellar; top phase liquid
A1	90	0	10	Bottom phase cubic; top phase liquid
A2	80	0	20	Bottom phase cubic; top phase liquid
A3	70	0	30	Bottom phase cubic; top phase liquid
A4	60	0	40	Bottom phase cubic, smaller in size; top phase liquid
A5	50	0	50	Bottom phase cubic, smaller in size; top phase liquid, emulsion present
A6	40	0	60	Bottom phase cubic, very small; top phase liquid; emulsion present
A7	33.3	0	66.7	Moderate viscosity emulsion
A8	25	0	75	Moderately low viscosity emulsion
A9	9	0	91	Low viscosity emulsion
B0	81	10	9	Single-phase isotropic liquid
B1	72.9	9	18.1	Bottom phase lamellar; top phase liquid
B2	64.8	8	27.2	Cubic with small amount of lamellar; top phase liquid
B3	56.7	7	36.3	Cubic phase; top phase liquid; viscous emulsion
B4	48.6	6	45.4	Cubic phase; top phase liquid; less-viscous emulsion
B5	40.5	5	54.5	Cubic phase; top phase liquid; less-viscous emulsion
C0	72	20	8	Single-phase isotropic liquid
C1	64.8	18	17.2	Lamellar phase plus excess liquid phase
C2	57.6	16	26.4	Cubic phase present; top phase liquid
C3	50.4	14	35.6	Cubic phase; top phase liquid; viscous emulsion
C4	43.2	12	44.8	Cubic phase; top phase liquid; less-viscous emulsion
C5	36	10	54	Cubic phase; top phase liquid; low-viscosity emulsion
D0	63	30	7	Single-phase isotropic liquid
D1	56.7	27	16.3	Lamellar phase (small) plus excess liquid phase
D2	50.4	24	25.6	Cubic phase present; top phase liquid
D3	44.1	21	34.9	Cubic phase; top phase liquid
D4	37.8	18	44.2	Cubic phase; top phase liquid; viscous emulsion
D5	31.5	15	53.5	Cubic phase; top phase liquid; low-viscosity emulsion
E0	54	40	6	Single-phase isotropic liquid
E1	48.6	36	15.4	Lamellar phase (very small) plus excess liquid phase
E2	43.2	32	24.8	Cubic and lamellar phases present; moderately high-viscosity emulsion
E3	37.8	28	34.2	Cubic and lamellar phases present; moderate-viscosity emulsion
E4	32.4	24	43.6	Low-viscosity emulsion
E5	27	20	53	Low-viscosity emulsion
F0	45	50	5	Single-phase isotropic liquid
F1	40.5	45	14.5	Almost entirely liquid; small amount of emulsion
F2	36	40	24	Moderate-viscosity emulsion with non-emulsified excess liquid phase
F3	31.5	35	33.5	Moderate-viscosity emulsion with non-emulsified excess liquid phase
F4	27	30	43	Low-viscosity emulsion
F5	22.5	25	52.5	Low-viscosity emulsion
G0	36	60	4	Single-phase isotropic liquid
G1	32.4	54	13.6	Almost entirely liquid; small amount of emulsion
G2	28.8	48	23.2	Moderate-viscosity emulsion with significant non-emulsified excess liquid phase
G3	25.2	42	32.8	Moderately-low viscosity emulsion
G4	21.6	36	42.4	Low viscosity emulsion
G5	18	30	52	Low viscosity emulsion

Table II

Original Samples from Table I before combination with Gentisic Acid					Subsequent combination with Gentisic Acid	
ID	Mix A (%)	Spear (%)	Water (%)	Description of phases present	Gentisic Acid added as % of total sample	Description of phases present after gentisic acid added
A0	100	0	0	Bottom phase lamellar; top phase liquid	10	No change *
A1	90	0	10	Bottom phase cubic; top phase liquid	10	No change *
A2	80	0	20	Bottom phase cubic; top phase liquid	10	No change *
A3	70	0	30	Bottom phase cubic; top phase liquid	10	No change *
A4	60	0	40	Bottom phase cubic, smaller in size; top phase liquid	10	No change *
A5	50	0	50	Bottom phase cubic, smaller in size; top phase liquid, emulsion present	10	No change *
A6	40	0	60	Bottom phase cubic, very small; top phase liquid; emulsion present	10	No change *
A7	33.3	0	66.7	Moderate viscosity emulsion	10	No change *
A8	25	0	75	Moderately low viscosity emulsion	10	No change *
A9	9	0	91	Low viscosity emulsion	10	No change *

* Excess gentisic acid crystals present

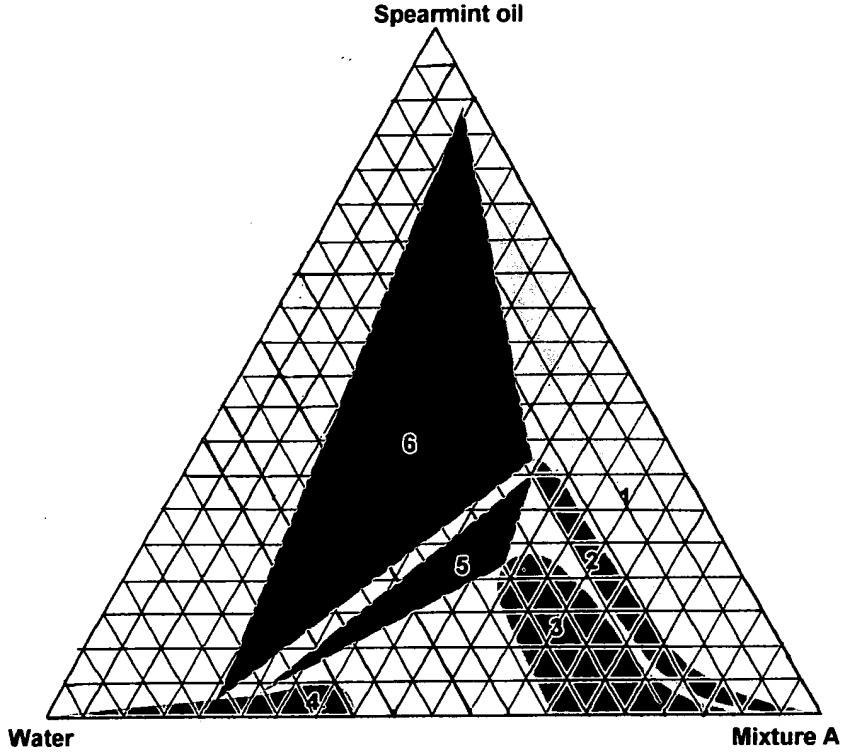
Table III - Comparison of
Reversed Cubic Phase and Reversed Hexagonal Phase Material Particles
with Oil-in-Water Emulsion

	Reverse Cubic Phase Material	Reverse Hexagonal Phase Material	Oil - in -Water Emulsion
			
Morphology	Long range nanometer-scale order. May be bulk material or particulate. Fluid lipid bilayer based, arranged with precise curvature in repeating cubic space groups, creating interlaced polar and apolar microdomains. Very high interfacial surface area.	Long range nanometer-scale order. May be bulk material or particulate. Fluid lipid bilayer based, arranged with precise curvature in repeating hexagonal space groups, creating polar microdomains. High interfacial surface area.	No long range nanometer-scale order. Fat droplets surrounded by lipid-rich layer, dispersed in water. Very low interfacial surface area.
Material phase composition	Reverse cubic phase material	Reverse hexagonal phase material	Oil-rich liquid phase surrounded by lipid-rich phase, dispersed in aqueous phase
Radius of monolayer Curvature*	3 nanometers	1.5 nanometers	100 nanometers
Specific Surface Area*	40 m^2/mL	25 m^2/mL	6 m^2/mL
Hydrophobic Volume Fraction*	50%	80%	95%
Farthest distance to polar or apolar domain*	3 nanometers	3 nanometers	98 nanometers
Load	Carries hydrophobic, hydrophilic, amphiphilic, and "schizophrenic" actives in hydrophilic domains, in hydrophobic domains, and straddling both domains	Carries hydrophobic, hydrophilic, amphiphilic, and "schizophrenic" actives in hydrophilic domains, in hydrophobic domains, and straddling both domains	Hydrophobic, fat-soluble actives only; Fat-soluble actives sequestered in core

* for comparison purposes, based on 200 nanometer diameter particles

Figure 1

Phase Diagram of Mixture A / Spearmint Oil / Water



The key to the regions labeled 1 – 6 is as follows:
Region 1: Oil-rich liquid phase.
Region 2: Primarily lamellar (Lα) phase, though excess oil generally present.
Region 3: Primarily normal cubic (V1) phase, though excess oil generally present.
Region 4: Primarily normal micellar (L1) phase, though excess oil generally present.
Region 5: Three phases present, lamellar, normal cubic, and normal micellar.
Region 6: Three phases present, lamellar, aqueous, and oil (typically emulsion-forming compositions).

Figure 2

Photomicrograph of Mixture A with the addition of gentisic acid

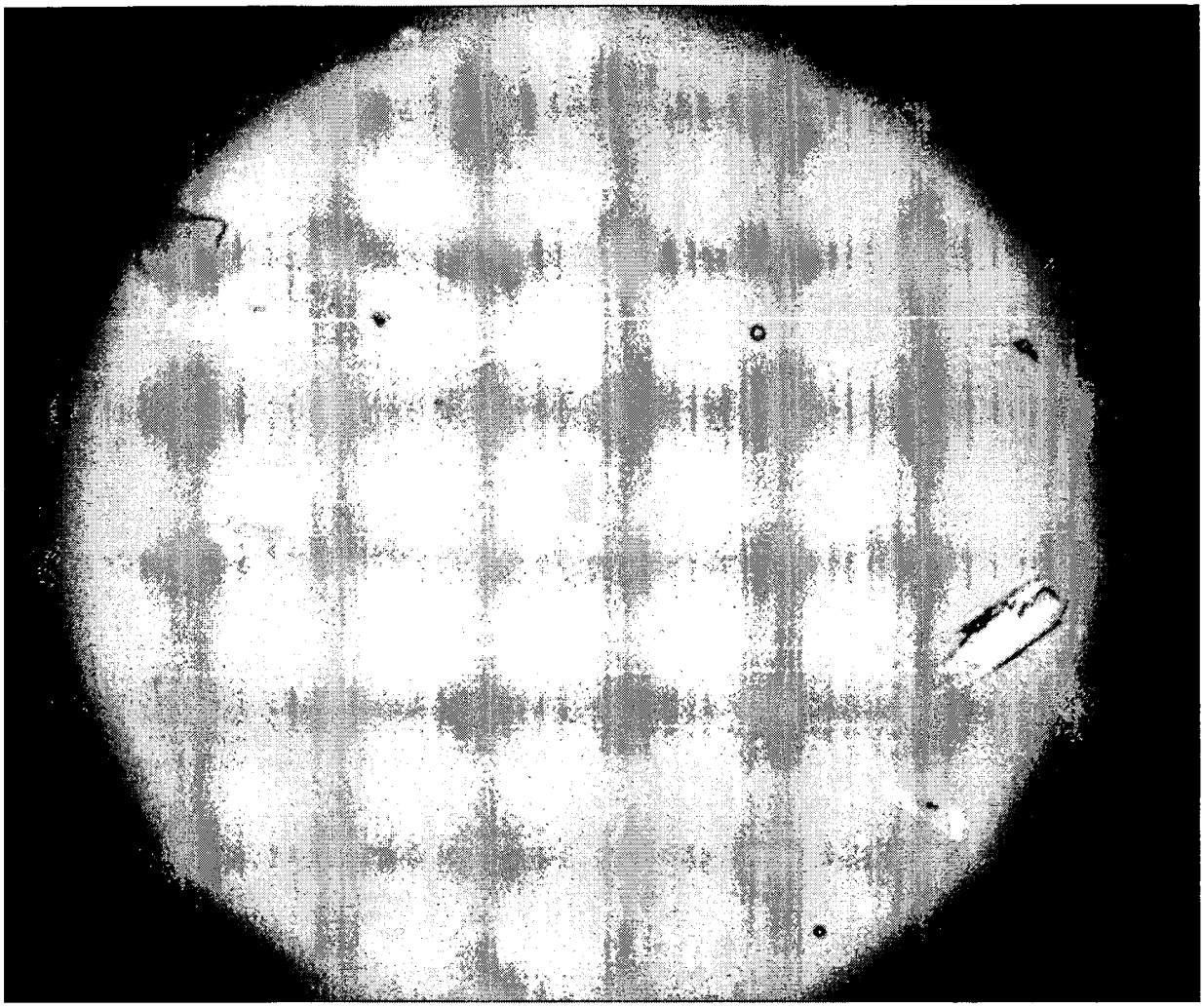
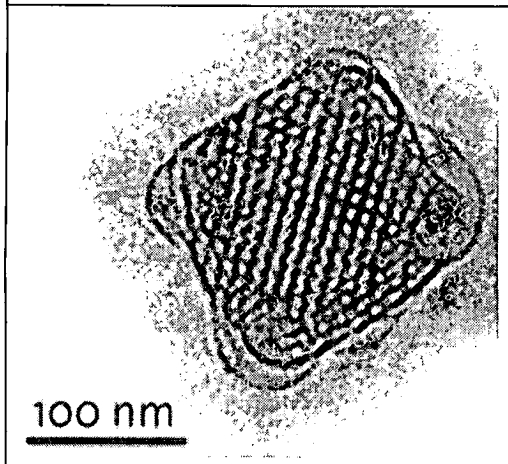
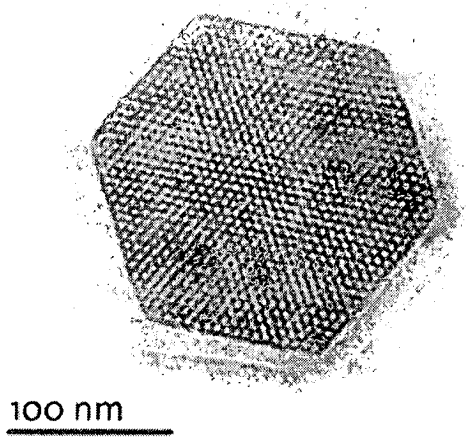
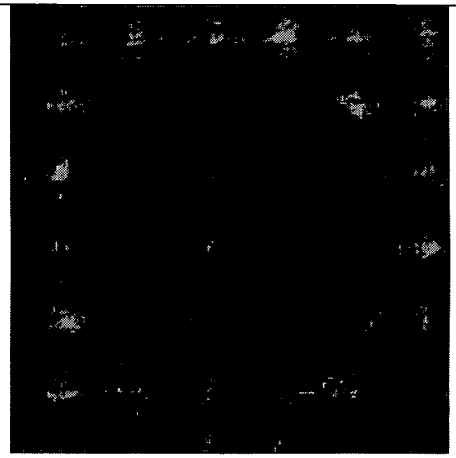


Figure 3

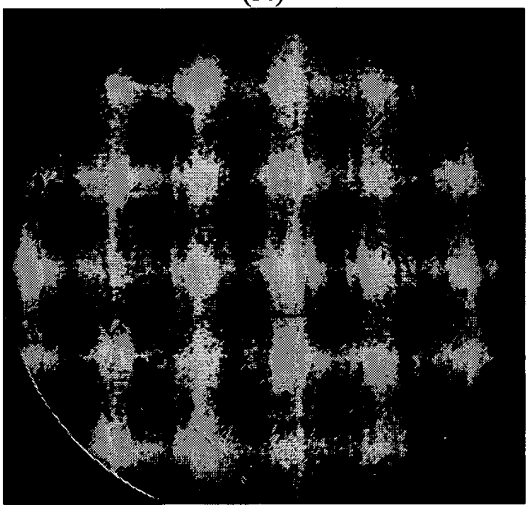
Electron Micrographs (TEM, TEM, TEM)

Cubic phase material	Hexagonal phase material	Emulsion droplet in Oil-in-Water Emulsion
		

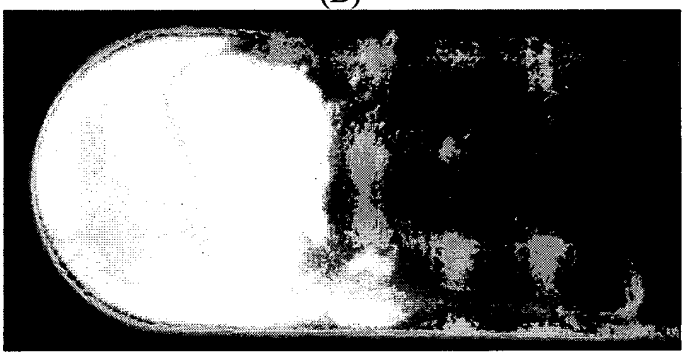
Attachment 1

Photomicrographs

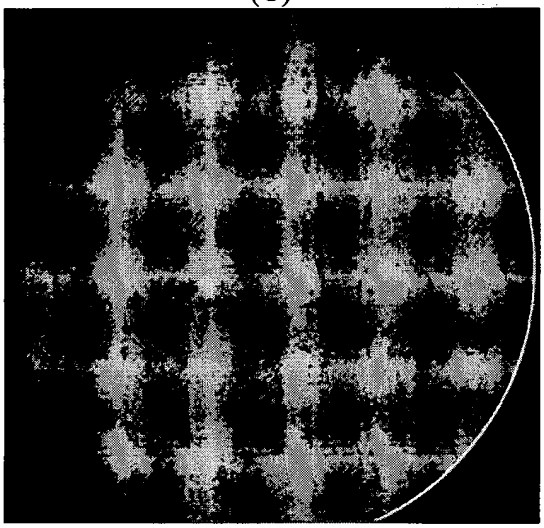
(A)



(B)



(C)



Attachment 2

Definitions

"Polar": polar compounds (such as water) and polar moieties (such as the charged head groups on ionic surfactants or on lipids) are water-loving or hydrophilic: "polar" and "hydrophilic" in the context of the present document are essentially synonymous. In terms of polar groups in hydrophilic and amphiphilic molecules (including but not limited to polar solvents and surfactants), a number of polar groups have been tabulated by Laughlin [Advances in liquid crystals, vol. 3. p. 41, (1978)], including a detailed discussion of which polar groups are operative as surfactant head groups and which are not.

"Apolar". An apolar compound is a compound that has no dominant polar group. Apolar (or hydrophobic, or alternatively, "lipophilic") compounds include not only the paraffinic/hydrocarbon / alkane chains of surfactants, but also modifications of them, such as perfluorinated alkanes, as well as other hydrophobic groups such as the fused-ring structure in cholic acid as found in bile salt surfactants, or phenyl groups as form a portion of the apolar group in Triton-type surfactants, and oligomer and polymer chains that run the gamut from polyethylene (which represents a long alkane chain) to hydrophobic polymers such as hydrophobic polypeptide chains in novel peptide-based surfactants that have been investigated. An apolar compound will be lacking in polar groups, a tabulation of which is included herein, and will generally have an octanol-water partition coefficient greater than about 100, and usually greater than about 1,000.

"Amphiphile": an amphiphile can be defined as a compound that contains both a hydrophilic and a lipophilic group. See D. H. Everett. Pure and Applied Chemistry, vol. 31. no. 6, p. 611 (1972). It is important to note that not every amphiphile is a surfactant. For example, butanol is an amphiphile, since the butyl group is lipophilic and the hydroxyl group hydrophilic, but it is not a surfactant since it does not satisfy the definition, given below. There exist a great many amphiphilic molecules possessing functional groups which are highly polar and hydrated to a measurable degree, yet which fail to display surfactant behavior. See Laughlin, op cit.

"Surfactant": A surfactant is an amphiphile that possesses two additional properties. First, it significantly modifies the interfacial physics of the aqueous phase (at not only the air-water but also the oil-water and solid-water interfaces) at unusually low concentrations compared to nonsurfactants. Second, surfactant molecules associate reversibly with each other (and with numerous other molecules) to a highly exaggerated degree to form thermodynamically stable, macroscopically one-phase, solutions of aggregates or micelles. In the present context, any amphiphile which at very low concentrations lowers interfacial tensions between water and hydrophobe, whether the hydrophobe be air or oil, and which exhibits reversible self-association into nanostructured micellar, inverted micellar, or bicontinuous morphologies in water or oil or both, is a surfactant.

"Polar-apolar interface": In a surfactant molecule, one can find a dividing point (or in

some cases two points, if there are polar groups at each end, or even more than two, as in Lipid A, which has seven acyl chains and thus seven dividing points per molecule), in the molecule that divide the polar part of the molecule from the apolar part. In any nanostructured liquid phase or nanostructured liquid crystalline phase, the surfactant forms monolayer or bilayer films: in such a film, the locus of the dividing points of the molecules describes a surface that divides polar domains from apolar domains: this is called the "polar-apolar interface" or "polar-apolar dividing surface." For example, in the case of a spherical micelle, this surface would be approximated by a sphere lying inside the outer surface of the micelle, with the polar groups of the surfactant molecules outside the surface and apolar chains inside it. Care should be taken not to confuse this microscopic interface with macroscopic interfaces separating two bulk phases that are seen by the naked eye.

"HLB: Hydrophilic-hydrophobic balance": With each surfactant is associated a number, generally between 0 and 20, which describes how hydrophilic the surfactant is—meaning of course, that it correlates with the aqueous solubility of the surfactant. In the original definition of HLB, the MW of the polar head was divided by the total MW of the surfactant molecule, and the resulting fraction multiplied by 20. Thus, a surfactant with an HLB of 10 has a polar head group that comprises 50% of the surfactant MW, to the extent that this definition is strictly maintained. In actual practice, assignment of the HLB for a particular surfactant is often done by the manufacturer, and often by running comparisons with well-established surfactants of known HLB. But in any case, the following is universally agree upon: highly polar surfactants with dominant head groups are assigned high HLBs greater than 10, while less polar surfactants with dominant hydrophobic groups (typically alkyl chains) are assigned low HLB values less than 10.

"Lamellar phase": The lamellar phase is a lyotropic liquid crystalline phase characterized by:

1. Small-angle x-ray shows peaks indexing as 1:2:3:4:5. . . in wave number.
2. To the unaided eye, the phase is either transparent or exhibits mild or moderate turbidity.
3. In the polarizing optical microscope, the phase is birefringent, and the well-known textures have been well described by Rosevear and by Winsor (e.g., Chem. Rev. 1968, p. 1). The three most pronounced textures are the "Maltese crosses", the "mosaic" pattern, and the "oily streaks" patterns. The Maltese cross is a superposition of two dark bands (interference fringes) roughly perpendicular to each other, over a roughly circular patch of light (birefringence), forming a distinctive pattern reminiscent of the WWI German military symbol. The variations on this texture, as well as its source, is thoroughly described in J. Bellare, Ph.D. Thesis, Univ. of Minnesota (1987). The "mosaic" texture can be envisioned as the result of tightly packing together a dense array of deformed Maltese crosses, yielding dark and bright patches randomly quilted together. The "oily streaks" pattern is typically seen when the (low viscosity) lamellar phase flows between glass and coverslip.

For lamellar phases in surfactant-water systems:

1. Viscosity is low enough so that the material flows (e.g. when a tube containing the phase is tipped upside down),

2. The self-diffusion rates of all components are high comparable to their values in bulk -- e.g., the effective self-diffusion coefficient of water in the lamellar phase is comparable to that in pure water.

3. If the surfactant is deuterated in the head group, and the ^{2}H NMR bandshape measured, one finds two spikes with the splitting between them twice what it is in the hexagonal phase.

4. In terms of phase behavior, the lamellar phase generally occurs at high surfactant concentrations in single-tailed surfactant / water systems, typically above 70% surfactant: in double-tailed surfactants, it often occurs at lower concentrations, often extending well below 50%. It generally extends to considerably higher temperatures than do any other liquid crystalline phases that happen to occur in the phase diagram.

"Normal (or Type I) hexagonal phase": The normal hexagonal phase is a lyotropic liquid crystalline phase characterized by:

1. Small-angle x-ray shows peaks indexing as 1:sqrt(3):2:sqrt(7):3 . . ., in general, sqrt($h^2 + hk - k^2$), where h and k are integers—the Miller indices of the two-dimensional symmetry group,

2. To the unaided eye, the phase generally transparent when fully equilibrated, and thus often considerably clearer than any nearby lamellar phase.

3. In the polarizing optical microscope, the phase is birefringent, and the well-known textures have been well described by Rosevear and by Winsor (e.g., Chem. Rev. 1968, p. 1). The most distinctive of these is the "fan-like" texture. This texture appears to be made up of patches of birefringence, where within a given patch fine striations fan out giving an appearance reminiscent of an oriental fan. Fan directions in adjacent patches are randomly oriented with respect to each other.

For normal hexagonal phases in surfactant-water systems:

1. Viscosity is moderate, more viscous than the lamellar phase but far less viscous than typical cubic phases (which have viscosities in the millions of centipoise).

2. The self-diffusion coefficient of the surfactant is slow compared to that in the lamellar phase: that of water is comparable to that in bulk water.

3. The ^{2}H NMR bandshape using deuterated surfactant shows a splitting, which is one-half the splitting observed for the lamellar phase.

4. In terms of phase behavior, the normal hexagonal phase generally occurs at moderate surfactant concentrations in single-tailed surfactant water systems, typically on the order of 50% surfactant. Usually the normal hexagonal phase region is adjacent to the micellar (L1) phase region, although non-bicontinuous cubic phases can sometimes occur in between. In double-tailed surfactants, it generally does not occur at all in the binary surfactant-water system.

"Reversed (or Type II) hexagonal phase": In surfactant-water systems, the identification of the reversed hexagonal phase differs from the above identification of the normal hexagonal phase in only two important respects:

1. The viscosity of the reversed hexagonal phase is generally quite high, higher than a typical normal hexagonal phase, and approaching that of a reversed cubic phase. And,

2. In terms of phase behavior, the reversed hexagonal phase generally occurs at high surfactant concentrations in double-tailed surfactant / water systems, often extending to, or close to, 100% surfactant. Usually the reversed hexagonal phase region is adjacent to the lamellar phase region which occurs at lower surfactant concentration, although bicontinuous reversed cubic phases often occur in between. The reversed hexagonal phase does appear, somewhat surprisingly, in a number of binary systems with single-tailed surfactants, such as those of many monoglycerides (include glycerol monooleate), and a number of nonionic PEG-based surfactants with low HLB.

"Normal (or Type I) bicontinuous cubic phase": The normal bicontinuous cubic phase is a lyotropic liquid crystalline phase characterized by:

1. Small-angle x-ray shows peaks indexing to a three-dimensional space group with a cubic aspect. The most commonly encountered space groups, along with their indexings are: la3d (#230), with indexing sqrt(6):sqrt(8):sqrt(14):4... Pn3m (#224), with indexing sqrt(2):sqrt(3):2:sqrt(6):sqrt(8)... and 1m3m (#229), with indexing sqrt(2):sqrt(4):sqrt(6):sqrt(8)...

2. To the unaided eye, the phase is generally transparent when fully equilibrated, and thus often considerably clearer than any nearby lamellar phase.

3. In the polarizing optical microscope, the phase is non-birefringent, and therefore there are no optical textures.

For normal bicontinuous cubic phases in surfactant-water systems:

1. Viscosity is high, much more viscous than the lamellar phase and even more viscous than typical normal hexagonal phases. Most cubic phase have viscosities in the millions of centipoise.

2. No splitting is observed in the NMR bandshape, only a single peak, corresponding to isotropic motion.

3. In terms of phase behavior, the normal bicontinuous cubic phase generally occurs at fairly high surfactant concentrations in single-tailed surfactant / water systems typically on the order of 70% surfactant with ionic surfactants. Usually the normal bicontinuous cubic phase region is between lamellar and normal hexagonal phase regions, which along with its high viscosity and non-birefringence make its determination fairly simple. In double-tailed surfactants, it generally does not occur at all in the binary surfactant-water system.

"Reversed (or Type II) bicontinuous cubic phase": In surfactant-water systems, the identification of the reversed bicontinuous cubic phase differs from the above identification of the normal bicontinuous cubic phase in only one respect. In terms of phase behavior, the reversed bicontinuous cubic phase is found between the lamellar phase and the reversed hexagonal phase, whereas the normal is found between the lamellar and normal hexagonal phases: one must therefore make reference to the discussion above for distinguishing normal hexagonal from reversed hexagonal. A good rule is that if the cubic phase lies to higher water concentrations than the lamellar phase,

then it is normal, whereas if it lies to higher surfactant concentrations than the lamellar then it is reversed. The reversed cubic phase generally occurs at high surfactant concentrations in double-tailed surfactant / water systems, although this is often complicated by the fact that the reversed cubic phase may only be found in the presence of added hydrophobe ("oil") or amphiphile. The reversed bicontinuous cubic phase does appear in a number of binary systems with single-tailed surfactants such as those of many monoglycerides (include glycerol monooleate) and a number of nonionic PEG-based surfactants with low HLB.

It should also be noted that in reversed bicontinuous cubic phases, though not in normal, the space group #212 has been observed. This phase is derived from that of space group #230.

"Normal (or Type I) discrete (non-bicontinuous) cubic phase": The normal non-bicontinuous cubic phase is characterized by:

1. Small-angle x-ray shows peaks indexing to a three-dimensional space group with a cubic aspect. The most commonly encountered space group in surfactant systems is Pm3n (#223) with indexing sqrt(2):2:sqrt(5).... In single-component block copolymers, the commonly observed space group is Im3m, corresponding to body-centered sphere-packings with indexing sqrt(2):sqrt(4):sqrt(6):sqrt(8) ....

2. To the unaided eye, the phase is generally transparent when fully equilibrated, and thus often considerably clearer than any associated lamellar phase.

3. In the polarizing optical microscope, the phase is non-birefringent and therefore there are no optical textures.

For normal discrete cubic phases in surfactant-water systems:

1. Viscosity is high, much more viscous than the lamellar phase and even more viscous than typical normal hexagonal phases. Most cubic phase have viscosities in the millions of centipoise, whether discrete or bicontinuous.

2. Also in common with the bicontinuous cubic phases, there is no splitting in the NMR bandshape, only a single isotropic peak.

3. In terms of phase behavior, the normal discrete cubic phase generally occurs at fairly low surfactant concentrations in single-tailed surfactant water systems, typically on the order of 40% surfactant with ionic surfactants. Usually the normal discrete cubic phase region is between normal micellar and normal hexagonal phase regions, which along with its high viscosity and non-birefringence make its determination fairly simple. In double-tailed surfactants, it generally does not occur at all in the binary surfactant -water system.

"Reversed (or Type II) discrete cubic phase": In surfactant-water systems, the identification of the reversed discrete cubic phase differs from the above identification of the normal discrete cubic phase in three respects:

1. In terms of phase behavior, the reversed discrete cubic phase is found between the lamellar phase and the reversed hexagonal phase, whereas the normal is found between the lamellar and normal hexagonal phases: one must therefore make reference to the discussion above for distinguishing normal hexagonal from reversed hexagonal. A

good rule is that if the cubic phase lies to higher water concentrations than the lamellar phase, then it is normal, whereas if it lies to higher surfactant concentrations than the lamellar then it is reversed. The reversed cubic phase generally occurs at high surfactant concentrations in double-tailed surfactant / water systems, although this is often complicated by the fact that the reversed cubic phase may only be found in the presence of added hydrophobe ('oil') or amphiphile. The reversed discrete cubic phase does appear in a number of binary systems with single-tailed surfactants, such as those of many monoglycerides (include glycerol monooleate), and a number of nonionic PEG-based surfactants with low HLB.

2. The space group observed is usually Fd3m. #227.

3. The self-diffusion of the water is very low, while that of any hydrophobe present is high; that of the surfactant is generally fairly high, comparable to that in the lamellar phase.

"Phase Diagram" is the tool routinely used by scientists in the field of lyotropic liquids and liquid crystals to record the lyotropic liquid or liquid crystal phase or composition structure which is created at each unique relative concentration of the same ingredients. Thus, in the figure set forth in Fennell and Wennerstrom at pp 501-505, different variations in the relative concentrations of octanol, Posassium caprate and water create alternatively micelles, reversed micelles, normal hexagonal phase, reversed hexagonal phase or lamellar phase materials. In Table 1 of this Declaration, different relative combinations of the Lambert mixture, spearmint and water yield different compositions including liquid, lamellar, normal cubic and normal micellar.

Attachment 3

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THE COLLOIDAL DOMAIN

Where Physics, Chemistry, Biology, and Technology Meet

Second Edition

D. Fennell Evans
and
Håkan Wennerström

WILEY-VCH

NEW YORK · CHICHESTER · WEINHEIM · BRISBANE · SINGAPORE · TORONTO

- The Gordon parameter ($\gamma/V^{1/3}$ N/m^2, where γ and V are the solvent's surface tension and molar volume) provides a useful measure of solvent cohesive energy, as illustrated in Figure 1.11.
- Micelle formation is observed in polar (water, hydrazine, and ethylene glycol, $1.3 < \gamma/V^{1/3}$) and in nonpolar (oil and fluorocarbons, $\gamma/V^{1/3} < 0.3$) solvents, but not in intermediate solvents (alcohols, esters, and amides).

1

Most colloidal phenomena involve a liquid phase as at least one of the actors. In this liquid we can typically identify a *solvent* and one or more *solutes*. Molecular interactions involving colloidal entities, solutes, and solvents determine the properties of the system.

Throughout this book we try to maintain this molecular perspective on the colloidal phenomena. In this chapter we apply this strategy to a description of the general characteristics of amphiphilic assemblies. As we shall see, these colloidal self-assembled structures arise from a delicate interplay between solute–solvent and solute–solute interactions. The term **amphiphilic** indicates that one part of the molecule likes the solvent, while the other does not.

1.1 Amphiphilic Self-Assembly Processes Are Spontaneous, Are Characterized by Start–Stop Features, and Produce Aggregates with Well-Defined Properties

Amphiphilic molecules spontaneously self-organize into a variety of structures. The simplest and best understood of these is the micelle. To characterize the amphiphilic aggregation process, we can begin by considering how adding surfactant to water leads to the formation of this typical structure.

One example of a dual-character molecule possessing the well-defined polar head and nonpolar tail needed to produce amphiphilic behavior is sodium dodecyl sulfate (SDS), whose structure is given in Table 1.1. In aqueous solutions up to 8×10^{-3}M, most of the properties SDS displays are similar to those we can observe for a typical electrolyte such as NaCl. Figure 1.1 illustrates an exception: decreasing surface tension, denoted as γ.

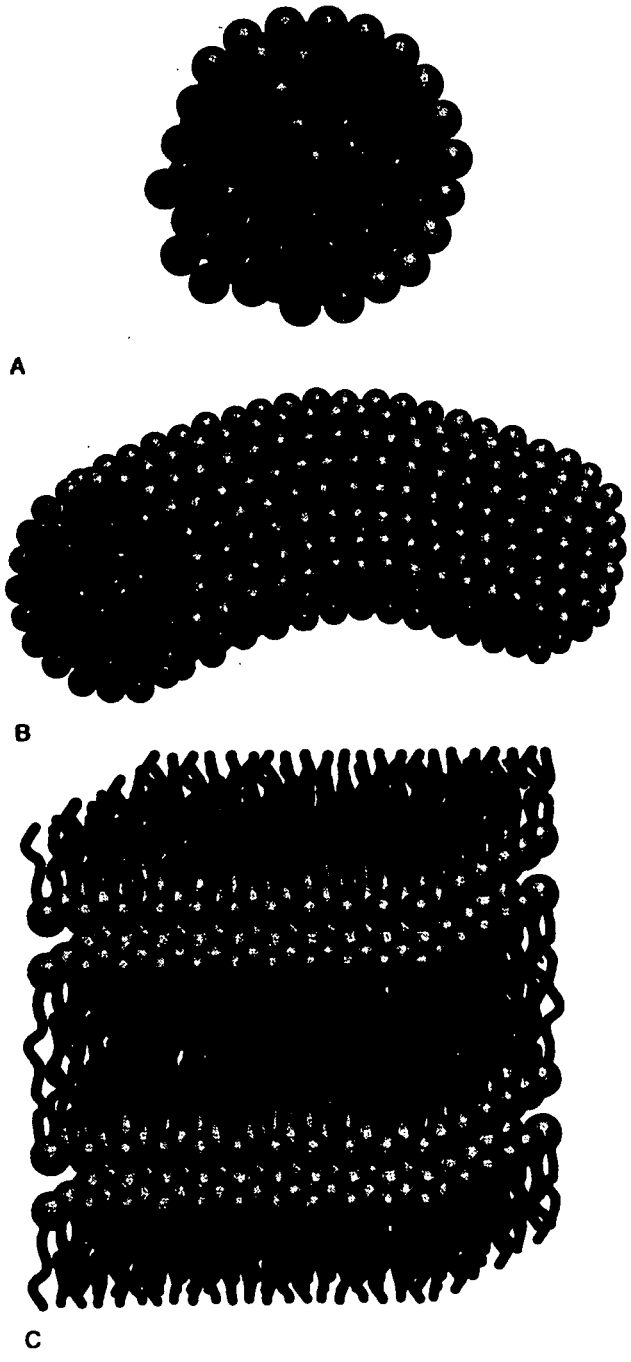
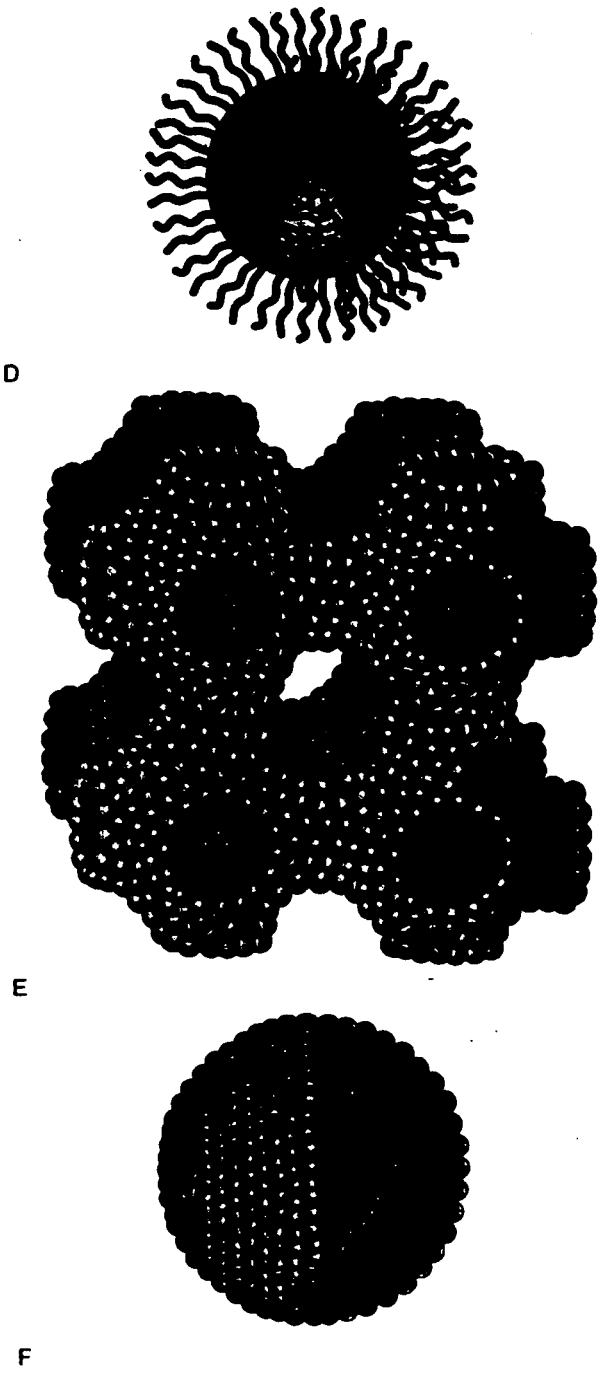


Figure 1.6
Amphiphilic aggregate structures. (A) Spherical micelles ($N_s = 0.33$) in which the micelle's radius $R_{micelle} \approx l_{max}$. For SDS micelles $R_{micelle} = 20$Å, the micelle aggregation number N is 60, and the head group area a is 60 Å^2. The actual cross- sectional area of the sulfate head group is 27 Å^2. Thus, 55% of the micelle's surface involves direct contact between hydrocarbon and water. (B) Cylindrical micelles ($N_s = 0.5$) in which $R_{micelle} = l_{max}$. The ends of the cylinder are capped by hemispheres covered by surfactant head groups. Cylindrical micelles are usually polydisperse because the rod can grow to varying lengths by simply incorporating more surfactants into the cylindrical portion of the micelle. (C) Planar bilayers ($N_s = 1$) in which the bilayer thickness is $\approx 1.6 l_{max}$ in the liquid state. Such structures can grow in the bilayer plane without limit and usually curve back on themselves to minimize exposure of hydrocarbons at the edges of the bilayer to water. (D) Inverted micelles ($N_s > 1$) in which the head groups point into an aqueous environment and the hydrocarbon tails point out into the continuous oil medium. Such inverted structures can be spheres, cylinders, or vesicles. (E) Bicontinuous structures ($N_s \geq 1$) in which the two radii of curvature are equal but of opposite sign, leading to a small mean curvature. (F) Vesicles ($N_s \approx 1$) formed by small regions of bilayers closing back on themselves to form a hollow spherical structure in which the interaqueous compartment is isolated from the surroundings.



group. Because amphiphiles with single hydrophobic chains have surfactant parameters of less than 0.5, they are constrained—at least in dilute solution—to form micellar aggregates. Adding a second hydrocarbon chain doubles v, while a_0 and l ramain essentially the same. In effect, the addition doubles v/a_0l into the range 0.5–1. Hence, double-chain surfactants tend to form bilayer structures such as vesicles and lamellar liquid crystals, whose curvatures are inherently lower than those formed by their single-chain counterparts.

A comparable, although quantitatively different, approach to analyzing aggregate structures used the curvature concept explicitly. In this approach, the crucial parameter is not the surfactant number N_s but the preferred mean curvature of a surfactant film. We can define the **mean curvature** H at a point on a surface as

$$H = \tfrac{1}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \qquad (1.3.8)$$

where R_1 and R_2 are the radii of curvature in two perpendicular directions, as illustrated in Figure 1.7. For a sphere, $R_1 = R_2 = R$ and $H = 1/R$, for a cylinder, $R_1 = R$, $R_2 = \infty$ and $H = 1/2R$, while for a planar bilayer, $H = 0$. A value of $H = 0$ also can occur on a saddle-shaped surface in which $R_1 = -R_2$. To assign a sign to the radii of curvature, we must define a normal direction, n, for the surface. By convention, $\vec{n}$ is usually positive, pointing toward the polar region, and therefore the curvature of inverted aggregates is negative.

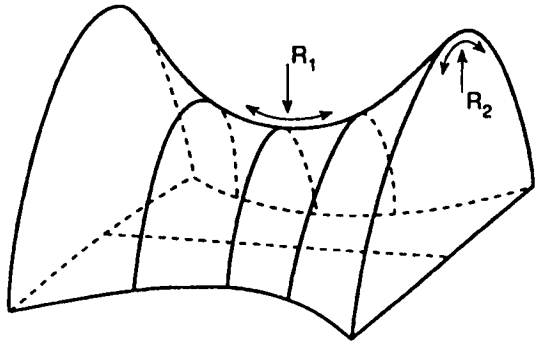
We will consider the specific properties of the various amphiphilic aggregates shown in Figure 1.6 in later chapters. The rest of this chapter deals with the general features of solution and solvents.

1.4 Understanding the Origin of Entropy and Enthalpy of Mixing Provides Useful Molecular Insight into Many Colloidal Phenomena

Throughout this book we will be concerned with the thermodynamics of the self-assembly of surfactant molecules into aggregates, the properties of polymer solutions, and the behavior of colloidal entities such as emulsions. To obtain a basis for these more complex systems we start by considering the mixing of two pure liquids. Figure 1.8 shows three possible outcomes of mixing two components:

1. Formation of a homogeneous solution resulting from complete miscibility at all compositions. An example is t-BuOH and water at 25°C.
2. Formation of two phases containing different concentrations of the two components. An example is n-BuOH and water at 25°C.

Figure 1.7
Radius of curvature. For a surface in three dimensions, two mutually perpendicular radii of curvature, R_1 and R_2, can be specified at each point. On a saddle-shaped surface, the two radii of curvature have opposite sign. Here R_1 and R_2 are shown at two different points on the surface.



3. Retention of two unmixed phases, illustrated by water and mercury.

In a thermodynamic description, the outcome of these mixing processes differs because the free energies of mixing, ΔG_{mix}, are different. At constant T and P, the Gibbs free energy is

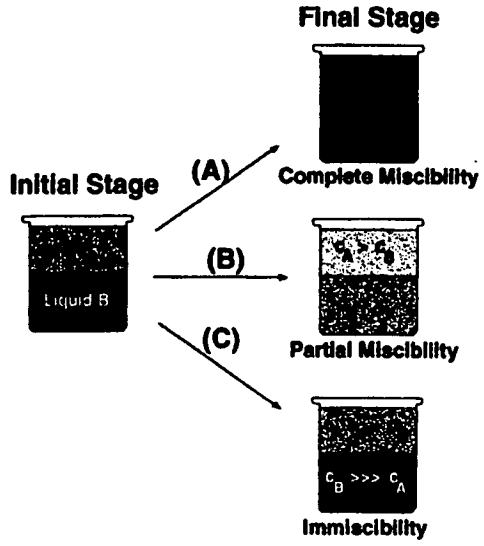
$$\Delta G = \Delta H - T\Delta S \qquad (1.4.1a)$$

and for a mixing process

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} \qquad (1.4.1b)$$

where $\Delta G_{mix} = G_{final\ solution} - G_{initial\ solution}$. We can define ΔH_{mix}

Figure 1.8
Three possible outcomes of mixing equal amounts of two liquids A and B: (a) Formation of a homogeneous mixture; (b) Formation of two phases, one solution of B in A and one solution of A in B; (c) retention of the two unmixed phases with the second component present in small amounts.



quantity determined by the length of the hydrocarbon chains while there is no molecular limit to bilayer growth in the lateral direction. In an ideal situation, bilayers attempt to minimize the degree to which the hydrocarbons at their edges are exposed to the aqueous environment by extending indefinitely in the lateral direction. In reality, finite samples show defects, and bilayers tend to close up on themseves, leading to a variety of macroscopic structures described in the next section.

In micellar solutions, the relaxation times for monomer exchange range from 10^{-3} to $10^{-6}\,s^{-1}$, and they involve a diffusion-limited process that depends directly on the monomer concentration. In bilayers, monomer exchange is considerably slower, as predicted by their much lower monomer solubility.

Lifetimes for typical micelles vary from 10^{-3} to 10^{-1} s. With bilayers, such transformations involve rearranging the entire bilayer structure, and a particular specific macroscopic state often reflects the methods used to prepare the sample. Thus, micelles respond rapidly to changes in their environment, while bilayers are much more sluggish. It can require hours, days, or even years for a bilayer to achieve an equilibrium state.

The transport properties of micelles and bilayers also display major differences. With spherical micelles, all directions are equivalent, and a surfactant molecule can diffuse very rapidly within a micelle. A bilayer, on the other hand, has two distinct diffusion modes. The first involves lateral diffusion within the bilayer plane. It occurs rapidly since it mainly involves the amphiphile chains moving through the oillike region of the bilayer. The second diffusional mode is slow since it involves moving the head group from one side of the bilayer to the other. This "flip-flop" requires the polar head group to rotate through the oillike interior of the bilayer. In pure bilayers, the time required to execute this flip-flop process is typically hours to days.

6.1.4 Pure Amphiphiles Form a Range of Bulk Bilayer Phases

The lamellar liquid crystal is the generic bulk phase of a bilayer-forming amphiphile. As illustrated in Figure 1.6c, a lamellar liquid crystal consists of a stack of bilayers separated by aqueous films. In the liquid crystalline state, the molecules diffuse freely in the lateral direction, while they move in the perpendicular direction only on a very long time scale. The apolar chains behave as in a liquid with a sizable fraction of gauche conformations. The forces acting between bilayers determine the thickness of the aqueous layer. As discussed in the previous chapter, a range of forces is operating, and bilayer systems are very useful in the study of these forces.

When a lamellar phase is cooled, it undergoes a phase change that can lead to the formation of a crystal or sometimes to a stable or metastable gel phase. Figure 6.3 shows some typical gel phase structures. Their characteristic feature is that the alkyl chains are crystallized while liquidlike solvent still

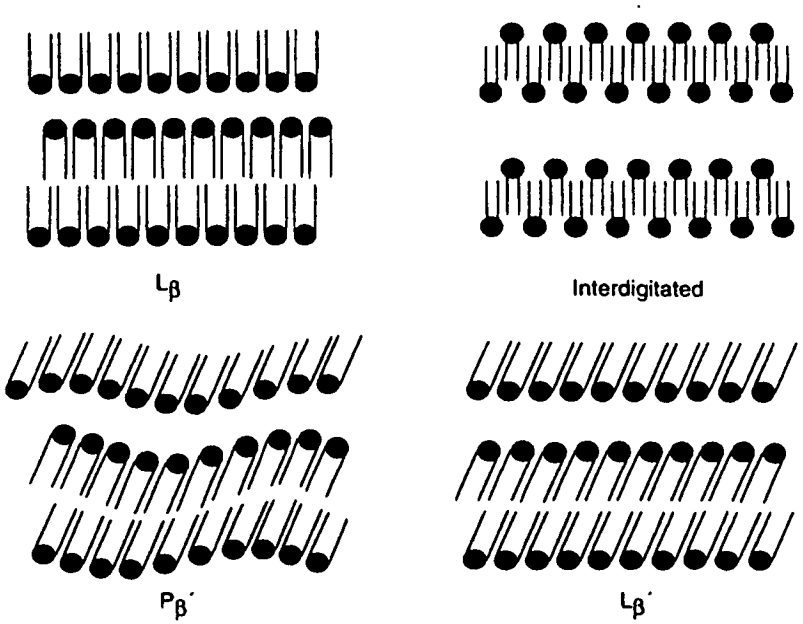


Figure 6.3
When alkyl chains crystallize, they have a cross-sectional area of $\approx 20\,Å^2$ per chain in a plane perpendicular to the direction of the chain. If the area of the polar groups in the bilayer matches that of the chain(s), a structure L_β is adopted. In the more likely case of a mismatch between polar head and chain areas, a tilted structure, as in L_β', or a rippled structure, as in P_β', can appear. For single-chain amphiphiles, chains may even interdigitate, making the thickness of the apolar layer the same as the length of a single chain.

exists between the bilayers. The crystalline state of the chains implies that more stringent conditions are imposed on the packing. In this case, a mismatch can easily arise between the cross-sectional area per chain and the head group area. To accommodate such a mismatch, the chains may tilt relative to the bilayer plane, the bilayer may develop a ripple, or the chains may even interdigitate, depending on the degree of mismatch.

Bilayers form the basic structural element in other ordered phases. Figure 6.4 shows a representative bicontinuous phase of cubic symmetry in which the bilayer is draped on a surface of zero mean curvature. Because the structure is bicontinuous, the amphiphile and the solvent each diffuse in three dimensions within its own medium, displaying an effective diffusion coefficient only slightly smaller ($\sim 2/3$) than its value in a homogeneous solution. Under these circumstances, the bilayer separates the solvent into two distinct regions. Molecular transport from one region to the other involves passage through the bilayer, a fact that has particular relevance for biological membrane systems.

In isotropic solutions, bilayers can appear in the form of vesicles shaped like spherical shells (see Figure 1f). Depending on the nature of these vesicles, we usually distinguish between small unilamellar vesicles (SUVs; radius $\lesssim 100$ nm), large unilamellar vesicles (LUVs), and multilamellar liposomes. There is no sharp distinction between liposomes and a lamellar phase with excess solvent. Vesicles tend to occur most readily for systems with surfactant numbers N_s slightly below one. They are seldom true equilibrium systems, but they are often meta-

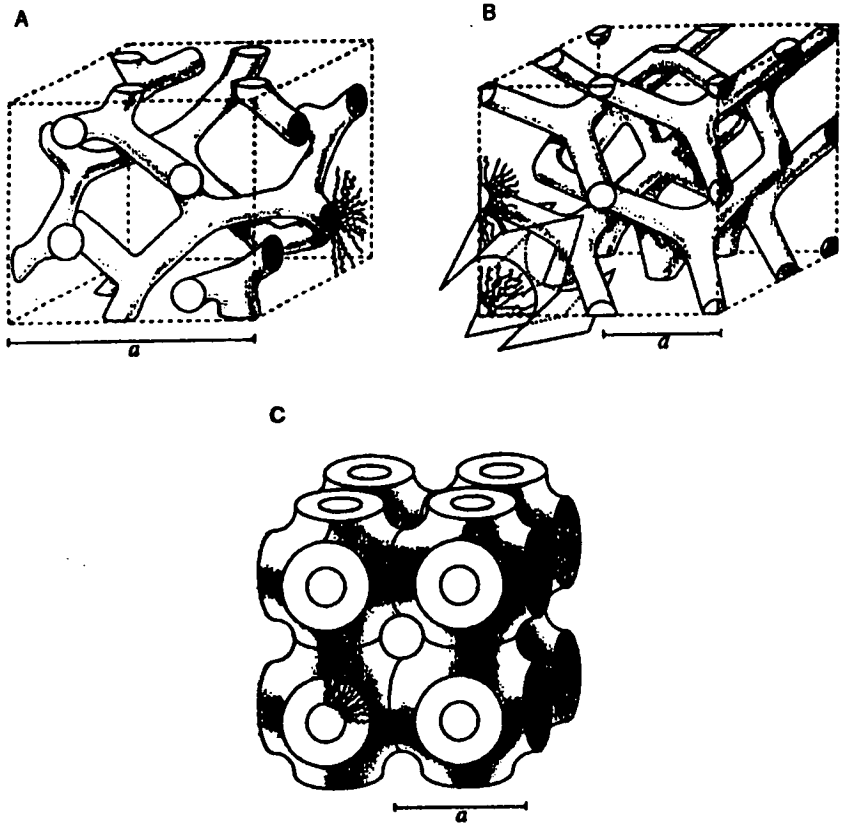


Figure 6.4
Illustration of three different arrangements of bilayers in cubic symmetry: (a) a gyroid structure; (b) a double diamond (as in two interwoven diamond lattices); and (c) plumbers nightmare with the same symmetry as in the monolayer version of Figure 1.6e. (Seddon, *Biochem. Biophys. Acta* **1031**, 1–69 (1990).)

stable enough to allow detailed physical and chemical studies that have proved very valuable. In vitro studies of membrane proteins are one example.

An intriguing bilayer arrangement commonly found in surfactant systems is the so-called sponge or L_3 phase. In this phase, the bilayer extends for an unlimited distance in three dimensions, forming a single macroscopic aggregate with isotropic properties. This phase corresponds to a disordered version of a cubic phase shown in Figure 1.6e and it occurs for surfactant numbers N_s slightly larger than one.

The difference in free energy between the various bilayer structures often is merely on the order of a small fraction of kT per molecule. Thus, small changes in temperature or composition can cause the transformation from one structure to another.

6.1.5 Vesicles Can be Formed by Several Methods

Vesicles commonly occur in the living cell and many physiological processes involve steps where vesicles are formed or consumed by fusion with another bilayer system. The importance of vesicles is not restricted to living systems and they are also very useful for in vitro studies of membrane physical and chemical properties. Because most vesicles are thermodynamically unstable, the properties of a vesicle solution depend on how it is prepared.

A simple but nonselective way of producing vesicles is to subject a lamellar dispersion to ultrasound, such as a lamellar phase containing excess water. The sound waves tear the lamellae, which then can reseal into vesicles. The smaller the vesicle, the less the perturbation affects it; so the result of the ultrasound treatment is a broad but skewed size distribution of vesicles that peaks at rather small aggregates, typically $R \simeq 150$—200 Å. A disadvantage of the sonication process is that the large energy input may chemically degrade the lipid.

Cholate dialysis represents a more controlled method for preparing vesicles. In this technique we prepare a dilute solution of lipid–cholate mixed micelles and dialyze it. High monomer solubility enables the cholate to readily pass the dialysis membrane. As a result, the cholate content of the lipid solution will decrease slowly but steadily, and the lipid content of the micelles will increase until the large aggregates finally close to form vesicles. These vesicles possess a more uniform size, which we can control by varying parameters such as salt content, temperature, and pH.

Another way of producing vesicles is to inject a solution of the lipid in an organic solvent or solvent mixture into an excess of water. Large unilamellar vesicles form as the organic solvent evaporates or is dissolved in the water. Because the experimental conditions can vary greatly, obtaining vesicles of specified properties with this method is to some extent an art rather than a science.

In many instances, vesicles appear "spontaneously" as we prepare the lipid–water system. We can obtain large unilamellar vesicles by simply taking a dry lipid powder and adding the aqueous solvent. With charged lipids, continuous dilution of the system can lead to the formation of vesicles under the action of double-layer repulsions.

The kinetic stability of a vesicle system depends on the rate of the process by which two vesicles fuse to form a larger one. Vesicle stability is really an example of the general problem of colloidal stability, discussed further in Chapter 8.

The fusion process is easier to understand if we think of it as occurring in two steps. In the first step, the respective bilayers come into proximity. The occurrence of this step depends strongly on interbilayer forces. For charged systems with low electrolyte concentrations, double-layer repulsion largely prevents two vesicles from meeting. For zwitterionic phospholipid vesicles, a sizable shortrange stabilizing force also tends to prevent close molecular contact.

The second step in the process actually fuses the two bilayers. Any substantial barrier to this process may cause the two vesicles to aggregate and stay together for long periods without ever fusing. Factors that influence the fusion process

surface tension—diverge/vanish as the independent variable, for example T, approaches a critical value, T_c.

The transformation of a gas–liquid two-phase system to a supercritical fluid at the critical point provides an example. As the physical differences between the two phases diminish and merge at T_c, long range molecular correlations become important.

Continuous transformations can be characterized by power law expressions with characteristic exponents (eqs. 10.4.1–10.4.4) that can be predicted from statistical mechanics. Surprisingly, the same set of critical exponents describes many seemingly diverse physical systems.

We can illustrate many features of continuous phase transitions using regular solution theory. The critical exponents are inaccurate, reflecting the failure of mean field theory to correctly account for long range molecular correlations.

Other Sources of Continuous Transformations

So far we have focused on macroscopic systems possessing a few well-defined components. Other sources of apparently continuous phase transformations include:

- Systems in which finite size effects play a dominant role, such as in the formation of small micelles leading to a broad CMC.
- Systems containing impurities or inhomogeneities, which broaden phase transformations.
- Systems in which equilibrium times are long compared to observation times, as in glass transitions for polymers.

10

In colloidal systems, interparticle or interaggregate interactions can easily be strong enough to promote macroscopic ordering that can lead to the formation of a sequence of phases. Slight variations in conditions may be enough to trigger phase changes that in turn induce major changes in macroscopic properties. Although surfactants and biological lipids display particularly rich behavior, the phenomenon is also important in other colloidal systems.

The study of phase equilibria fulfills a dual purpose. As the most important factor in the thermodynamic characterization of a system, phase behavior often provides an essential clue to macroscopic behavior. In addition, we can link phase behavior in an intimate but nontrivial way with molecular or particle interactions. If we understand this connection properly, we can control phase behavior by changing interactions on a molecular level. Conversely, we can study interaggregate interactions by observing phase behavior.

10.1 Phase Diagrams Depicting Colloidal Systems Are Generally Richer Than Those for Molecular Systems

10.1.1 Several Uncommon Aggregation States Appear in Colloidal Systems

Colloidal systems show a greater diversity in aggregation states than we find in molecular systems. Reviewing the general terminology used to describe different states and phases can help us appreciate this fact, but we must remember that whenever we attempt to systematize a complex physical reality, we encounter borderline cases that may not fit a given classification system.

At ambient temperatures, matter, exists in a solid, liquid, or gaseous state or in a mixture of these. Dynamic properties at the molecular level provide the basis for characterizing aggregation states. In a **solid**, a molecule's translational motion is slow to very slow. In a **liquid**, molecules (or colloidal particles) diffuse quite rapidly in space and interact constantly with their neighbors. In a **gas**, a molecule may travel unperturbed over a distance relatively large compared to its size before colliding with another molecule.

These three main aggregation states display a range of phase types, each with its own specific characteristics. For example, the most common form of solid is a **crystal**, in which molecules are arranged with long range positional and orientational order. Closer to the melting point, a crystal can undergo a phase transition, becoming noticeably softer. It maintains full positional order in this **plastic crystal** state, but it loses some orientational order and molecules rotate in their lattice sites. This behavior commonly occurs for compounds with long hydrocarbon chains.

In **amorphous solids**, molecules (or atoms) are not located in precisely determined lattice sites, as they are in crystals. An important example—found commonly in solid polymers but also seen in other colloidal systems—is a **glass**. The molecular arrangements we observe in glasses are very similar to those found in liquids, while the local dynamic properties are typical of solids. Amorphous solids often represent nonequilibrium structures, but because of the slow dynamics in the solid state, they can remain unchanged for indefinite times.

Although molecules diffuse rapidly in a liquid, in principle, a long range order can be maintained. However, for ordinary **isotropic liquids** only a short range order exists between neighboring molecules, and no correlations exist between molecules farther away. Most substances melt from a crystalline solid to an isotropic liquid. This change implies a transition from a very ordered state to a totally disordered one.

However, a number of compounds show a more gradual melting behavior, with one or more transitions between the crystalline solid and the isotropic liquid. These intermediate phases or **liquid crystals**, retain some, but not all, of the macroscopic order present in crystal, while there is a high molecular mobility.

Liquid crystalline phases that are induced by temperature changes are denoted as thermotropic liquid crystals. There is a considerable chemical diversity among the substances forming thermotropic liquid crystals. The most well known type is the one containing a stiff elongated aromatic backbone with a short alkyl chain at the ends. However, compounds like cholesterol esters, pure soaps, and some polymers can also form liquid crystals. In colloidal systems we are normally dealing with two or multicomponent systems, with particles or aggregates in a solvent. In this case, one uses the term lyotropic liquid crystals. The thermotropic and lyotropic liquid crystals differ somewhat in the chemical interactions causing the formation of the ordered phases, but the thermodynamic properties are basically the same.

In a **smectic** liquid crystal, molecules show long range (macroscopic) orientational order and also long range translational order in one or two directions. The term **smectic**, which is derived from the Greek word for soap, sometimes refers specifically to a layered structure like those found in the lamellar liquid crystals discussed in Chapter 6, but other forms of positional ordering are also possible like the hexagonal or cubic phases we have mentioned in Chapter 1. Figure 10.1 illustrates a typical molecular ordering in a thermotropic smectic phase.

When a smectic phase is heated, it can melt into an isotropic liquid. More often, however, it transforms into a phase in which the long range positional order has disappeared, but long range orientational order remains. The nature of this orientational order depends on whether the molecules are optically active (chiral).

A nonchiral molecule produces a **nematic** liquid crystalline phase, as illustrated in Figure 10.2, in which the preferred orientation is homogeneous throughout the system. Because

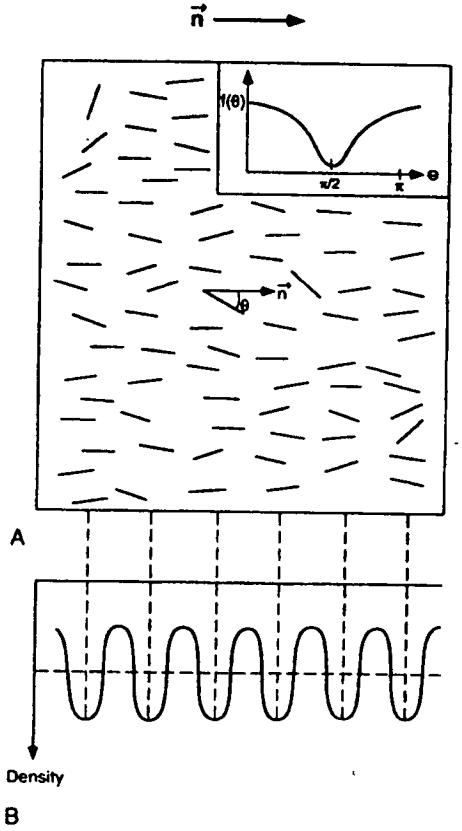


Figure 10.1
Illustration of molecular order in a smectic phase. The molecules are preferentially oriented along the direction *n*. Acareful examination also reveals a layering in the direction of *n*. When the layering and the orientation lie along the same direction, *n*, the phase is called smectic *A*, otherwise it is called a smectic *C* phase. (A) The orientational distribution function $f(\theta)$ with maxima at $\theta = 0, \pi$ and a minimum at $\theta = \pi/2$. (B) The center-of-mass density along the director showing a periodic variation defining the layer thickness.

The total amount of matter is constant so that

$$dn_1^\beta = -dn_1^\alpha \quad \text{and} \quad dn_2^\beta = -dn_2^\alpha \tag{10.1.6}$$

Thus,

$$dG = (\mu_1^\alpha - \mu_1^\beta)dn_1^\alpha + (\mu_2^\alpha - \mu_2^\beta)dn_2^\alpha \tag{10.1.7}$$

and at the minimum, $dG = 0$, that can only occur for

$$\mu_1^\alpha = \mu_1^\beta \quad \text{and} \quad \mu_2^\alpha = \mu_2^\beta \tag{10.1.8}$$

because n_1^α and n_2^α can vary independently. In the general case,

$$\mu_i^\alpha(X^\alpha) = \mu^\beta(X^\beta) \tag{10.1.9}$$

for all components i, where X^α and X^β denote the compositions of the two phases α and β, respectively.

By definition

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,p,n_j} \tag{10.1.10}$$

so we can calculate the chemical potential if we know G as a function of the composition. The intercept method provides a convenient way to graphically determine μ_1 and μ_2 for two-component systems. It is particularly useful for determining qualitative features.

Figure 10.4 illustrates the method through a plot of $G/(n_1 + n_2)$ versus X_1 (or X_2). We obtain the chemical potentials

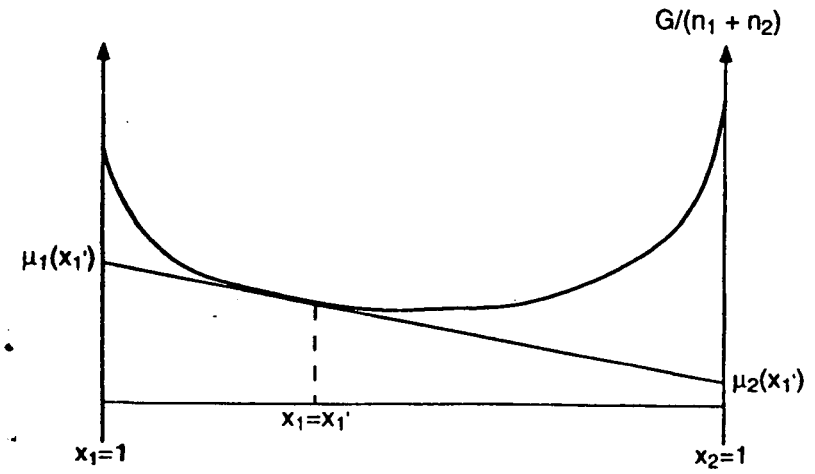


Figure 10.4
The intercept method for determining chemical potentials. Plot free energy G over total amount $(n_1 + n_2)$ versus concentration. The chemical potentials at a composition X_1' are obtained by constructing the tangent to the $G/(n_1 + n_2)$ curve at the particular concentration. The two chemical potentials, $\mu_1(X_1')$ and $\mu_2(X_1')$ are then obtained as the intercepts at $X_1 = 1$ and $X_2 = 1$, respectively.

at an arbitrary composition X_1' by taking the tangent of the curve, $G/(n_1 + n_2)$, at X_1'. The intercepts of this tangent at $X_1 = 1$ and $X_2 = 1$ are the chemical potentials $\mu_1(X_1')$ and $\mu_2(X_1')$. (For proof see any text on physical chemistry.)

With two components, there are three independent intensive variables. At fixed T and p, only one is left. Now the chemical potentials, μ_1, and μ_2, are both intensive. Thus, they cannot be varied independently at constant T and p. The interdependence between the two is expressed in the Gibbs–Duhem equation

$$n_1 d\mu_1 = -n_2 d\mu_2 \tag{10.1.11}$$

If we measure the concentration dependence of the chemical potential of one component, we can calculate the concentration dependence of the other by using eq. 10.1.11. This explains, for example, how we can use a measurement of the osmotic pressure—that is, the chemical potential of the solvent—to study interactions between solute molecules or colloidal particles.

10.1.4 Phase Diagrams Conveniently Represent Phase Equilibria

Phase diagrams provide us with a useful visual way to represent phase behavior. In one diagram we can summarize a large body of experimental data, and we also can use the phase diagram for predictive purposes.

A two-component system may have up to three degrees of freedom, but we can display only two dimensions conveniently in a graphic representation. Pressure usually influences condensed phases only slightly. In such a case, we decrease the number of degrees of freedom by one by holding the pressure constant (e.g., at 1 atm), while we vary temperature T and composition X.

Figure 10.5 illustrates some general features of T–X (p fixed) diagrams through a generic case of two components A and B, which form separate solid phases, yet show miscibility in the liquid state l. For one-phase regions with two degrees of freedom ($f = 2$), T and X vary independently, and the phase remains the same, although it exhibits continuously varying properties.

When two phases are in equilibrium, $f = 1$. In the diagram, these regions also have a two-dimensional character, but changes in total composition do not change the individual composition of the two phases in equilibrium. Their only effect is to change the relative amount of each phase. For example, a system containing one mole at composition X and temperature T (such as the one illustrated in Figure 10.5) will separate a moles of solid A and b moles of solid B in such a way that $a + b = 1$.

Conservation of component 1 implies

$$X_2' = aX_2^A + bX_2^B \rightarrow a(X_2^A - X_2') = b(X_2' - X_2^B) \tag{10.1.12}$$

which is called the **lever rule.**

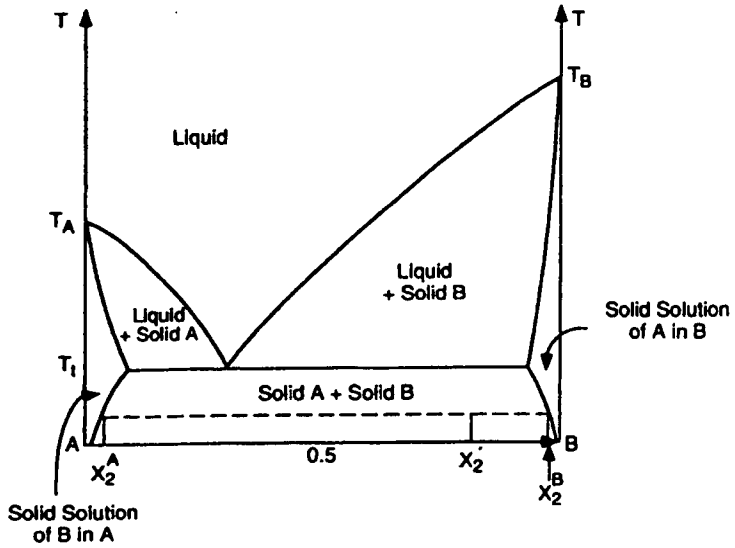


Figure 10.5
Binary T–X phase diagram showing one-phase and two-phase areas and a three-phase line at $T \simeq T_t$. Use of the lever rule in a two-phase area is also shown. The diagram represents the system Ag(A)–Cu(*B*).

The horizontal straight line at temperature T_t is called a three-phase line. The three phases, *A*, *B*, and *l*, are in equilibrium at this temperature, and consequently $f = 0$. No degree of freedom exists, and both the temperature and the compositions of the phase in equilibrium are unique.

Systems with three components may have as many as four degrees of freedom. To represent the phase behavior of such a system in a two-dimensional diagram, we must fix two degrees of freedom, usually T and p. Then we can represent the composition in a triangular diagram. As an example, Figure 10.6 shows the phase equilibria for a water–potassium decanoate ($C_9H_{19}COOK$)–octanol system.

Figure 10.7 demonstrates how to represent the composition by means of such a diagram. Each corner represents a pure component, while the opposite base of the triangle represents the binary system of the other two components. We arrive at the relative amount of component *A* at an arbitrary point *P* by drawing a line parallel to the *A* base. As shown in Figure 10.7, the intersection of the parallel line with the sides of the triangle determines the fraction of *A*.

The phase diagram divides the triangle into three types of area. One-phase areas have two degrees of freedom and can take any shape. Two-phase areas may have four corners joined by two opposing straight lines and two opposing curved lines, or we can replace the straight line(s) by concave lines ending in a critical point. These phases have only one degree of freedom, which shows that variations in one direction do not change intensive variables. In a complete phase diagram, we mark the

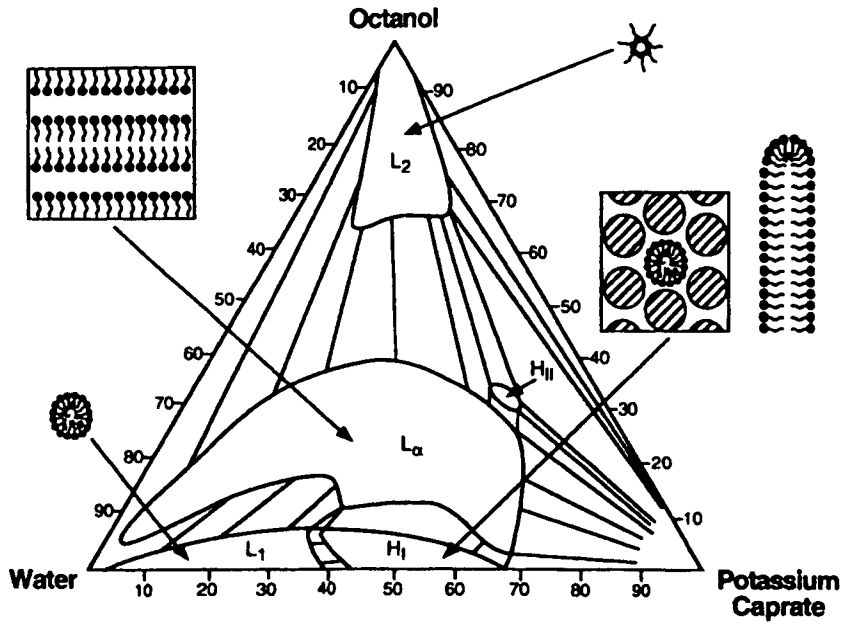


Figure 10.6
Phase diagram of the ternary system water–potassium decanoate (captrate)–octanol at constant pressure (1 atm.) and constant temperature (20°C). L_1 indicates a micellar and L_2 a reversed micellar solution. H_I is a normal hexagonal and H_{II} a reversed hexagonal liquid crystalline phase; L_α is a lamellar phase. (B. Jönsson and H. Wennerström, *J. Phys. Chem.* **91**, 338 (1987).)

invariant direction by so-called tie lines, which connect points on the two opposing curved lines as exemplified in Figure 10.6. Tie lines connect one-phase points in equilibrium. Only the proportions of the two phases change for points along the same tie line, and the lever rule (eq. 10.1.12) applies.

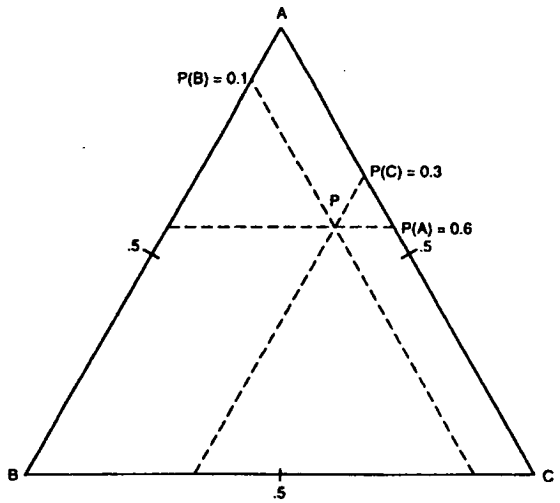
Finally, for three phases in equilibrium, $f = 0$. This situation occurs in so-called three-phase triangles. For a point within such a triangle the three phases represented by the corners of the triangles coexist in equilibrium. The relative amounts are determined in the same way as explained in connection with Figure 10.7, except that we are no longer dealing with an equilateral triangle.

10.1.5 Determining Phase Equilibria Is a Demanding Task

Determining the phase equilibria or phase diagram is usually the most important characterization of the macroscopic properties of a colloidal system. In general, determination of a complete phase diagram involves a lot of work. The basic principle is to mix the components and observe the number and nature of the phases. However, this can be done in a number of ways, depending on the particular characteristics of the sample.

The most obvious, direct, and often the most versatile method, is direct visual observation, be it by the naked eye or

Figure 10.7
Evaluating the composition at a point P in a triangular diagram. The relative amounts of A, B, and C are 0.6, 0.1, and 0.3, respectively.



in a polarizing microscope. Isotropic liquid phases typically phase separate readily, and mixing at a given composition and analyzing the amount and composition of the different phases in equilibrium can make it possible to determine a tie line. So when phases readily separate macroscopically, determining the phase diagram is a straightforward task.

However, colloidal systems that abound in liquid crystals and other viscous phases require a long time for achieving a macroscopic separation of the sample into two distinct regions with a single planar interface. It is more common that phases in equilibrium are dispersed in one another with domain sizes of tens or hundreds of micrometers. For each prepared sample, we face three questions:

How many phases are present?

What is the nature of the phases (liquid, liquid crystal, or solid) and what is the symmetry?

What are the compositions of the phases (if more than one phase is present)?

If macroscopic phase separation does not exist, we are forced to perform measurements on the whole sample and obtain some sort of macroscopic average. The three most common approaches are calorimetry, (x-ray) scattering, and spectroscopic (NMR, ESR, fluorescence) measurements.

Calorimetric measurements are useful only for making a T–X diagram. Differential scanning calorimetry (DSC) readily measures the heat capacity as a function of the temperature, T. By repeating the measurement for a range of compositions, we can localize the temperatures of three-phase lines and of

melting transitions. (See, e.g., Figure 6.18). However, phase changes that involve only small changes in enthalpic properties may remain undetected.

Small-angle x-ray or neutron scattering is sensitive to the structure of the phase. Thus, it is very useful for determining the nature of a phase, although it is more difficult, but still possible, to use the method when several phases are present.

Spectroscopic methods rely on the fact that spectroscopic properties are often sensitive to the local molecular environment. Thus, the same molecule in different phases will give different spectroscopic responses. In a multiphase sample, this behavior can be very useful, making it possible to count the number of phases in a straightforward way. Figure 10.8 shows as an example a series of deuterium NMR spectra used to construct the phase diagram of the system dipalmitoylphosphatidylcholine–water shown later (in Figure 10.21). By measuring the number of signals, their intensity, and the magnitude of the quadruple splittings, the combined information more than suffices to construct the phase diagram.

We conclude this section by noting that some occasions require a rapid method for determining phase behavior. It is no coincidence that such methods have been developed at the scientific laboratories of the two leading detergent producers in the world, Procter & Gamble and Unilever. A common principle is to take a surfactant crystal, place it in a microscope between coverslips, and let water penetrate into the crystal from one side by a diffusion process. During the diffusion process, a concentration gradient is generated and, in principle, the whole compositional range can be sampled across the coverslip. By focusing the (polarizing) microscope at different positions along the concentration gradient, one can determine the sequence of phases. Phase boundaries appear as sharp lines, making it relatively easy to detect a change in phase structure.

10.2 Examples Illustrate the Importance of Phase Equilibria for Colloidal Systems

A phase transition or an equilibrium between two phases typically occurs when a balance exists between two macroscopic states: one more ordered (with a low energy and entropy) and one less ordered (with a higher energy and entropy). In colloidal systems, interactions between particles can easily be of order kT and higher, yet entropies of mixing N_A and N_B objects are the same irrespective of the size of the objects. Consequently, ordered phases occur more readily in colloidal than in small molecule systems. This observation is particularly true for self-assembling systems whose intricate interplay between inter- and intra-aggregate interactions results in a particularly rich phase behavior. We will discuss a number of colloidal systems in which phase behavior constitutes an important aspect of the properties of the system in general.

Attachment 4

CV / Biographical Sketch: David M. Anderson

Lyotropic Therapeutics, Inc., 10487 Lakeridge Pkwy, Ashland, VA

EDUCATION

Ph. D. Chemical Engineering, University of Minnesota, June 1986.
Advisors: H. Ted Davis and L. E. Scriven; Enhanced Petroleum Recovery/Surfactant Microstructures Group.
Thesis: "Studies in the Microstructure of Microemulsions".

M. S. Mathematics, University of Minnesota, May 1982.

WORK HISTORY

1999 – present: **V.P. Scientific Affairs** at Lyotropic Therapeutics, Inc. Leader of research effort focusing on novel drug-delivery systems, interactions between lipid systems/microparticles and biological systems, and non-lamellar biomembrane systems. In charge of design, production, and scale-up of new microparticle formulations, and animal testing in collaboration with CRO's and industrial partners.

1995 - 1999: **Principal Scientist** at SelectRelease, L.C. Developed oral controlled-release formulations based on synergistic combinations of lipids/surfactants and novel crystalline and polymeric coating materials. Particular focus on formulations for intestinal release. Pharmaceutical formulation work with photodynamic therapy agents led to successful animal tests demonstrating sustained release leading to tumor necrosis. Experimental work and oversight of research and development activities, analysis and report/proposal writing, with input into strategic planning, intellectual property and contracting, and technology acquisition. The pharmaceutical rights to the technology were sold to Lyotropic Therapeutics in 1999.

1991 - 1995: **Assistant Professor, Biomaterials Dept. and Department of Oral Surgery, SUNY Buffalo**; also adjunct faculty member (Research Assistant Professor) in Biophysics and Chemistry Depts. Research centered around controlled-release materials, and nanoporous polymers and hydrogels incorporating a wide range of polymers including novel polymerizable lipids and surfactants. Taught polymer, biophysics, and biomaterials courses. On the faculty of the NSF Industry/University Center for Biosurfaces (IUCB) which focuses on issues of biocompatibility, bioadhesion, tissue compliance, and biofilm characterization.

1987 - 1991: **Guest Researcher, Univ. of Lund, Sweden**, with Hakan Wennerström and Björn Lindman in Physical Chemistry 1 Dept., a world-renowned department in colloid and interface science. Research focused on lyotropic liquid crystals, and the polymerization thereof. Funded by NFR (Swedish NSF) and by STU (Swedish Board of Technical Development) for first 2 years; after that I independently funded by arrangements with various industries including Costar, Corp., Union Carbide, and Pharmacia LKB. Supervised one graduate student (Ph.D. Thesis March 1992).

1986 - 1987: **Post-doctoral fellow with the Polymer Science and Engineering and the Mathematics Departments of the Univ. of Massachusetts at Amherst**, with E. L. Thomas and David Hoffman. Research focused on block copolymers, primarily the modeling of thermodynamics and morphology in complex 3-dimensional microstructures.

1982 - 1986: **Director of X-ray Scattering Facility at the Univ. of Minnesota**. While a graduate student, responsible for all matters concerning the operation of the lab, which houses a Siemens D-500 Diffractometer with computer interfacing, and a modified Kratky small-angle camera with a position-sensitive detector.

OTHER SKILLS AND AWARDS.

Awards and societies: Graduation with high distinction; Tau Beta Pi, Phi Kappa Phi; Runner-up in 1993 Niagara Frontier Inventor of the Year Award; Finalist in 1997 Richmond's New Technology of the Year; Runner-up in 1988 "Innovation Cup" invention contest sponsored by a Swedish technical newspaper (Dagens Industri); member Controlled Release Society, American Association of Pharmaceutical Scientists (AAPS), and American Chemical Society (ACS);

Strathmore's Who's Who 2004-2005.

Instrumentation skills. TEM/SEM, ultrafiltration (including hardware/system design), small- and wide-angle X-ray diffraction, pulsed-gradient NMR, aerosol monitoring, particle characterization with light scattering as well as aerosol techniques, polarizing, fluorescence and DIC optical microscopy, IR, NMR (chemical shifts), UV, electrophoresis and liquid chromatography. Aerosol generation and characterization expertise includes condensation particle counters, differential mobility analyzers, electrospray nebulizers, together with a strong background in the characterization of submicron/nanoscale particle characterization via a range of techniques.

Mathematics/modeling/computer skills. Computer skills range from PC's to mainframes, including state-of-art supercomputer graphics dating back to the earliest days of computer graphics. Sophisticated finite element analyses, using mainframes and supercomputers, including 3D graphics representations of solutions. Analytical and FE solutions of flow patterns, diffusion profiles, scattering/diffraction phenomena, etc. Modeled structure-property relations in polymers. Image analysis including maximum entropy methods, which were extended to Fourier space in one of my publications. Modeling of thermodynamics, microstructures, spectroscopic and other measurements of complex structures. Engineering calculations, some CAD, and statistical/error analysis in support of mechanical design, construction and testing, including the invention, design and construction of the Integrated Virus Detection (IViD) system. PC skills include Word, WordPerfect, Excel, Quattro Pro, Netscape Composer and image processing programs such as Photoshop, PhotoImpact, Corel Draw, AnimationWorks, etc. Constructed Pentium 166MMX system from motherboard and components. Area of concentration in M.S. Mathematics was Probability and Statistics.

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E. L. Thomas, D. M. Anderson, C. S. Henkee, D. Hoffman, "Periodic area-minimizing surfaces in block copolymers", **Nature** 1988, 334, 598-601.

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D. M. Anderson, "A new technique for studying microstructures: 2H NMR bandshapes of polymerized surfactants and counterions in microstructures described by minimal surfaces", Supplement to **J. Physique**, Proceedings of Workshop on Geometry and Interfaces, Aussois, France, Sept. 1990, C71-1 - C7-18.

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D. M. Anderson and E. L. Thomas, "Morphology of star diblock copolymers in the strong-segregation limit", **Macromolecules** 1988 21, 3221-3230.

D.M. Anderson, **U.S. Patent** No. 5,244,799 (issued 1993) and associated European Patent #0292145, "Microporous materials."

D.M. Anderson, **U.S. Patent** No. 5,238,613 (issued 1993) "Preparation of polymeric hydrogel containing micropores and macropores for use as a cell culture substrate."

C.H. Wick and D.M. Anderson, **U.S. Patent** No. 6,051,189 (issued 2000) "System and method for detection, identification and monitoring of submicron particles".

D.M. Anderson, **U.S. Patent** Nos. 6,482,517 (issued 2002), 6,638,621 (issued 2003), and 6,989,195, and associated international filings, "Coated particle and methods of making and using the same".

D.M. Anderson, **U.S. Patent** No. 6,991,809 (issued 2005) "Particles with improved solubilization capacity", and associated international filings.

Additional book contributions: Geometric Analysis and Computer Graphics, ed. P. Concus, #17 MSRI Series, Springer-Verlag, 1990; Lectures in Minimal Surfaces, J. C. C. Nitsche, Springer-Verlag; NSF Mosaic, "Computer Images in Five Dimensions", ed. W. Kornberg, 1988; Chemical & Engineering News, Aug. 1985; and Islands of Truth, Ivars Pearson, 1992.

TEACHING.

Courses taught.include graduate courses in Biomaterials, Biophysics, and Polymers (University at Buffalo). This included the development of a new graduate Polymers course.

Student supervision includes one PhD and two M.S. students (completed theses/degrees).

SELECTED FUNDING AWARDS/SOURCES.

(Won project funding of over $1MM from 1987 – 1997): Industry/University NSF Center for Biosurfaces, Life Technologies, Inc., NSF, National Institutes of Drug Abuse (NIDA), US Army CBDCOM, Defense Advanced Research Projects Agency (DARPA), Proctor & Gamble, Swedish Board of Technical Development, Pharmacia LKB, Union Carbide Linde Division, Costar/Nuclepore Co., Phlo Corp.. Bausch & Lomb, Inc.

PRESENTATIONS.

National / International meetings.

American Physical Society meeting, Pittsburgh, PA, March 1994, invited talk.

Scientific Conference on Chemical and Biological Defense Research, Aberdeen Proving Ground, MD, Dec. 1994, invited talk.

Asilomar Conference on Polymers, Monterey, Cal., Feb. 1993, invited talk.

"Workshop on Ordering in Fluids", Amsterdam, Neth., Sept., 1990, invited talk.

"Geometry and Interfaces", Aussois, Fr., Sept. 1990, invited talk.

"Liquids at Interfaces", Les Houches, Fr., June 1989, invited talk.

European Colloid and Interfaces Society annual meeting, Arcachon, Fr., Sept. 1988; poster.

"Complex and Supermolecular Fluids", Exxon Corporate Research, July 1985, poster.

Society of Rheology Meeting, Knoxville, Nov. 1984, invited talk.

Society of Pure and Applied Mathematics, Seattle, July 1984, invited talk.

Microscopy Society of America annual meeting, Cincinnati, August 1993, invited talk.

Oak Ridge Conference of the American Association of Clinical Chemists "Pushing the Envelope II", April 2005, invited talk.

Industrial and government labs. Invited talks at industrial sites including:

Costar	Nuclepore	Sepracor	Donaldson	Union Carbide
Minntech	Amoco Chemicals	Exxon	Chevron	Xerox
Osmonics	Calspan	Procter & Gamble	3M	Schleicher & Schuell
Perstorp AB	Pharmacia	Laser Photonics	Gibco	Life Technologies
Mitretek	Centers for Disease Control		Surface Science Institute (Stockholm)	
Smith-Kline-Beecham		Ross Products	NaPro Biopharmaceutics	
Antex Biologics	Baxter Healthcare	Elan/Nanosystems		

Defense Department. Invited lectures at the Naval Research Laboratories and Army Edgewood Arsenal (1994 and 1995 Annual Conferences on Chemical & Biological Defense Research), in addition to the DoD-sponsored Asilomar and Chem/Biol Defense conferences cited above. Also site-visited for a block grant proposal I P.I.'d, with a 5-year budget of $2.1 million, selected as one of the top 3 among 227 competitive proposals.

University at Buffalo. Invited talks in the Departments of Biophysics, Oral Biology, Chemical Engineering, Chemistry, and the Roswell Park Cancer Institute; also the Western New York Science Forum and the NSF co-sponsored "Nanobiology at Interfaces" symposium.

Other universities. Invited talks at: Princeton (Physics Dept., at the invitation of Sol Gruner, and Chemical Engineering / Princeton Materials Institute, at the invitation of Bob Prud'Homme); Cornell (Physics, Stanislas Leibler); MIT (Materials Science and Eng., Edwin Thomas); James Madison University (Biotechnology Association); Umea Univ (Biochemistry, Goran Lindblom); U. Lund (Chemical Technology, Bertil Tornell); U. Arizona (Biochemistry, David O'Brien); U. Wash. Seattle (Chem. Eng., Eric Kaler); U. Michigan (Materials Science, David Martin); McMasters (Biochemistry, Materials Research Center); Medical College of Virginia (Division of Neurosurgery, Timothy VanMeter).

THE PHYSICS OF LYOTROPIC LIQUID CRYSTALS

PHASE TRANSITIONS AND STRUCTURAL PROPERTIES

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1

LYOTROPIC SYSTEMS: MAIN EXPERIMENTAL FACTS AND TECHNIQUES

1.1 Introduction

Liquid crystals [1] are intermediate states of matter or mesophases, halfway between an isotropic liquid and a solid crystal. In nature, some substances, or even mixtures of substances, present these mesomorphic states. This picture leads to the concept of *ordering*. In a solid crystal, the basic units display translational long-range order, with the center of mass of atoms or molecules located on a crystal lattice; in some cases, the basic units also display orientational order. In an isotropic liquid, the basic units do not present either positional or orientational long-range order. From one ordering limit (solid crystal) to the other (isotropic liquid), there may exist many different situations. In plastic crystals, the basic units (globular molecules, e.g.) are located on a lattice but without any orientational order. In liquid crystals, the basic units display orientational order and even positional order along some directions. These materials flow like an isotropic fluid and have characteristic optical properties of solid crystals. Liquid crystals were firstly classified as thermotropics and lyotropics, depending on the physico-chemical parameters responsible for the phase transitions.

In *thermotropic* liquid crystals the basic units are molecules, and phase transitions depend on temperature and pressure. A pronounced shape anisotropy (in other words, the anisometry) is the main feature of the molecules which give rise to a thermotropic mesophase. Rods, disks, and banana-shaped are examples of molecular geometries associated with thermotropic liquid crystals. Besides pure substances, mixtures of molecules can also present thermotropic mesomorphic properties. Thermotropics are widely used in displays of low energy cost and in many sensor devices.

Lyotropic liquid crystals, shortly called *lyotropics* or *lyomesophases*, are mixtures of amphiphilic molecules and solvents at given temperature and relative concentrations. The mesomorphic properties change with temperature, pressure and the relative concentrations of the different components of the mixture. An important feature of lyotropics, turning them different from thermotropics, is the self-assembly of the amphiphilic molecules as supermolecular structures, which are the basic units of these mesophases. Although there are not many devices based on lyotropics, their physico-chemical properties have an interesting interface with biology, and the understanding of these properties has been relevant for improving some technological aspects of cosmetics, soaps, food, crude oil recovery, and detergent production.

and the alkyl chain is perpendicularly attached to the core [12]. In the presence of polar and non-polar solvents, these molecules form lyotropic mesophases, with nanosegregation properties [12,13].

As a final remark, it is important to note that a polar group is not always required to be hydrophilic (nor is a non-polar group always hydrophobic). The topology of the molecule and its insertion into the water network is also important to characterize the solubility in water [14].

1.1.3 *Definition of a lyotropic mixture*

Under suitable conditions of temperature and relative concentrations, mixtures of amphiphilic molecules and solvents can give rise to a *lyotropic mesophase*. In this type of system, amphiphilic molecules form self-assembled super-structures of several shape anisotropies and sizes.

Let us firstly classify lyotropics into three big families:

(a) Micellar systems, with molecular aggregates, called *micelles*, of small shape anisotropy, as sketched in Fig. 1.3(a). These micelles are aggregates of amphiphilic molecules, with typical dimensions of about 10 nm and shape anisotropy of order 1 : 2 in linear dimensions.

(b) Systems with aggregates of large shape anisotropy, of typical order 1 : 100 in terms of linear dimensions. These aggregates are sometimes called *infinite*, but we do not use this nomenclature. In Fig. 3(b), we sketch a long cylindrical aggregate.

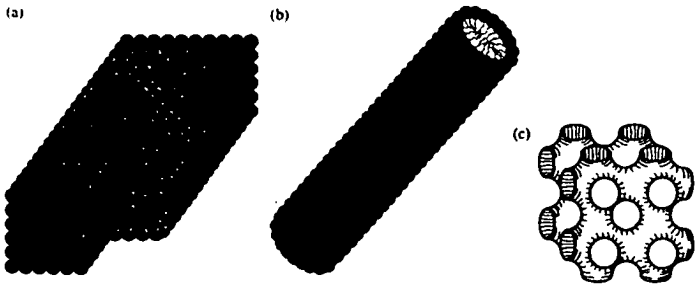

FIG. 1.3. Amphiphilic molecular aggregates. The polar head and the paraffinic chain of the molecules are represented by a sphere and a line, respectively: (a) sketch of an orthorhombic micelle. The cut in the right-down side shows the paraffinic chains in its inner part; (b) large anisotropic cylindrical aggregate; (c) sketch of a bicontinuous molecular aggregate with a cubic symmetry.

3

PHASE DIAGRAMS OF LYOTROPIC MIXTURES

3.1 Introduction

We have already emphasized that polymorphism is one of the main features of lyotropic liquid crystals. Phase diagrams of lyotropics, in terms of temperature and relative concentrations of the components, display a wealth of stable structures and regions. Changes of pressure can also lead to stable lyotropic phases [1]. In this chapter, we initially review some general features of phase diagrams, with emphasis on phase stability and the Gibbs phase rule. We then describe the topology of the best-known and investigated phase diagrams of binary, ternary and multi-component lyotropic mixtures. Finally, we refer to phenomenological calculations for obtaining some features of these phase diagrams.

3.2 General features of phase diagrams

The thermodynamic state of equilibrium of an isotropic system with r components is characterized by the internal energy U, the volume V, and the number of moles of each component, $N_1, N_2, \ldots, N_r$ [2]. The entropy $S = S(U, V, N_1, \ldots, N_r)$ is a homogeneous function of first degree of these variables. We then write the entropy per mole,

$$s = \frac{1}{N} S = s(u, v, x_1, \ldots, x_{r-1}), \tag{3.1}$$

where $u = U/N$ and $v = V/N$, with $N = N_1 + \cdots + N_r$, are the energy per mole and the specific volume, respectively, and $x_i = N_i/N$ is the molar relative concentration of component $i = 1, \ldots, r$. Note that

$$x_1 + x_2 + \cdots + x_r = 1, \tag{3.2}$$

and that we need only $r+1$ variables to fully characterize the equilibrium thermodynamic state of a simple r-component system. Usually, it is more convenient to work in the Gibbs representation, in which temperature T and pressure p, instead of u and v, are taken as the independent variables. For example, in the Gibbs representation, with $r = 2$ components (let us call them A and B), we need three independent variables (besides T and p, we may choose the relative molar concentration $x_A = N_A/(N_A + N_B)$ of component A).

We now consider a composite system of several simple subsystems (see Fig. 3.1). At given values of temperature and pressure, if the walls of this composite system are not restrictive, the chemical potential associated with each

DYNAMICS of SURFACTANT SELF-ASSEMBLIES

Micelles, Microemulsions, Vesicles, and Lyotropic Phases

I. INTRODUCTION

In the presence of water, surfactants and lipids give rise to a variety of phases referred to as lyotropic phases or mesophases.[1,2] The most important of these phases are the lamellar, hexagonal, cubic micellar, and cubic bicontinuous phases denoted by L, H and V, and Q, respectively (see Figure 1.11 in Chapter 1). The subscripts 1 or 2 attached to these phase symbols indicate that the phase is direct (water continuous) or inverse (discontinuous water domains). Many other lyotropic phases have been identified that differ from the main ones by the state of the alkyl chain (crystalline or disordered) and of the head group arrangement (ordered or disordered).[1] In the particular case of the lamellar phase, additional variations come from the possible different orientations adopted by the alkyl chains with respect to the plane of the lamellae (angle of tilt of the chain) and also from the state of the surface of the lamellae that can be planar or rippled.[1] Numerous detailed descriptions have been given for the equilibrium state of the various phases that surfactants and lipids can form in the presence of water.[1-3]

However, relatively few studies have addressed the dynamics of phase transitions of lyotropic phases of surfactants and lipids. The superb book *The Aqueous Phase Behavior of Surfactants*, by R.G. Laughlin,[3] has one chapter dealing with this topic. This chapter is 8 pages long and contains only 25 references, not all dealing with the dynamics of phase transitions, in a book of 558 pages. Likewise, the recent review on the kinetics of phase transitions in surfactant solutions by Egelhaaf[4] includes a rather short

paragraph on the kinetics of phase transitions between lyotropic phases. In addition, a literature survey shows that the majority of the reported studies on the dynamics of lyotropic phase transitions concern lipids and not surfactants. Many of these studies deal with the transformation of lipid lamellar phases into other phases and the reverse processes. The main reason for this situation is that the bilayers making up a lamellar phase are a good model for the cell membrane. Therefore, many studies of the rate of lipid phase transitions were undertaken in order to better understand the behavior of biological cells during replication as well as cell membrane lysis and reconstitution. As Laggner et al.[5] pointed out, studies of dynamics of phase transitions may provide "methods for prolonging the lifetimes of eventual structural intermediates in the transitions. A benefit from such an achievement could be the better structural description of the intermediates.... Also they are likely to be biomedical benefits from such results since the development of agents modulating the dynamics of membrane transformation, such as fusion, is likely to play an important role in many medical applications, e.g., liposome-based gene therapy, fertility modulation, or percutaneous drug applications." Most of the reported studies aimed at bringing information on the time scale of the transitions and on the nature of eventual intermediate phases between the known initial and final phases, and presenting mechanisms that account for the observations.

The various lyotropic phases can transform one into another when changing the temperature T[1-3] or the pressure p[6] applied to the investigated system, or the surfactant/lipid concentration C[1-3] in the system. Therefore, transitions between phases will be affected by changes in T, p, or C. This sensitivity provides a way to approach the dynamics of phase transitions in systems containing lipids or surfactants by means of T-jump, p-jump, and stopped-flow methods (see Chapter 2, Section II).

In the most recent studies of the kinetics of phase transitions, the investigated system is enclosed in a dedicated cell to which can be applied a p-jump, a T-jump, or a con-

The Lyotropic State of Matter

Molecular Physics and Living Matter Physics

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1 INTRODUCTION

1.1. LYOTROPIC LIQUID CRYSTALS

The lyotropic liquid crystal state of matter appears when some organic solid crystals dissolve in water or other polar (as well as non-polar) solvents and when solute concentration is high enough. Comparison to the thermotropic liquid crystal state is instructive: with thermotropic liquid crystals, initial solid crystal ordering is partially destroyed by raising temperature; with lyotropic ones this happens at ambient temperature due to the solvent-crystal interaction. The resulting highly concentrated solution displays a partially retained translational and orientational order (typical for the crystal solid) and partially acquires the properties of the isotropic solvent. The thus obtained anisotropic liquid contains at least two different molecular species. It is denoted by the following synonymous terms: "lyotropic liquid crystal", "lyotropic mesophase", "lyomesophase", "lyotropic", etc. The easiest way to detect the appearance of a lyotropic state in a given solution is to reveal its anisotropic optical properties (*viz.*, birefringence) by the method of polarizing microscopy. Naturally, all experimental methods of condensed matter physics capable of detecting anisotropic molecular ordering or anisotropic molecular diffusion can be successfully employed for the identification and structural characterization of lyotropic liquid crystals.

This condensed state of matter is typical for concentrated solutions of soaps (higher fatty acid salts), artificial washing products (surfactants, detergents), etc., but also for the solutions of a great

occurrence of mesophases is indicated by the apperance of the familiar J-aggregates' absorption band.

2.1.7. Hydrophilic–Lipophilic Balance

Depending on the relative contribution of their two parts, biphilic substances can take different positions on a scale, ranging from fully hydrophobic to fully hydrophilic substances. This position could be quantified by an empirical number called hydrophilic–lipophilic balance (HLB) varying on a conventional scale from 1 to 40 (Griffin, 1949, 1954; Davies & Rideal, 1963). Lyomesophases are most easily formed by those biphilic molecules with well expressed and balanced properties of their parts (HLB around 7). Rusanov (1991) has given a very useful interpretation of HLB-numbers in terms of the energy of transfer of the amphiphile $W^{\alpha\beta}$ between the two phases (water α and oil β) (which can be experimentally determined from the equilibrium distribution ratio of the bulk concentrations (c) of the amphiphiles in the two phases $W^{\alpha\beta} = k_B T \ln(c^{\alpha}/c^{\beta})$):

$$\mathrm{HLB} = 7 + 0.36(W^{\alpha\beta}/k_B T). \tag{2.1}$$

The coefficients 7 and 0.36 are somewhat arbitrary and related to the empirical character of HLB scale.

In the next section the complicated mesomorphism of the above-mentioned mesogens will be discussed.

2.2. POLYMORPHISM OF LYOTROPICS. BASIC TYPES OF MOLECULAR ORDERING

2.2.1. Lipid Monolayer. Hydrophobic Effect

The lyotropic polymorphism of a given mesogen comprises various types of lyomesophases that arise in a given solvent depending on concentration and temperature. As noted at the beginning, solvents can be polar (water, ethylene glycol, etc.) or non-polar (octane, decane, benzene, etc.). The mesomorphism in non-polar solvents should be called "lypotropic". As a rule, its appearance requires the presence of a certain amount of water in the system. Therefore, with a sufficient degree of generality one can consider a ternary system: polar phase/amphiphile/non-polar phase (e.g., water/soap/oil). Water and oil do not mix and an interface (or interfaces, if oil is dispersed) forms between them.

According to their bifunctional character, amphiphile molecules localize at the interface orienting their hydrophilic groups towards water and their lypophilic groups towards oil. In this manner a self-organized monomolecular layer of unidirectionally oriented amphiphiles emerges at the interface (Fig. 2.7.a). This type of monolayer arrangement of molecules is a basic one for lyotropics. We shall discuss the polymorphism regarding the inherent physical properties of the monolayer, its geometry (flat, curved) and topology (open, closed). Various monolayer configurations are sketched in Fig. 2.7. If one of the phases (polar or non-polar) is absent, it could be assumed to be

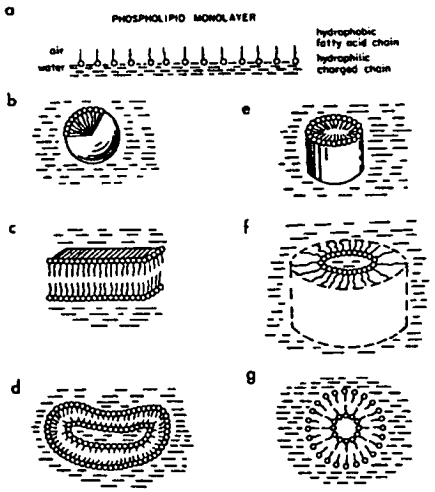

Figure 2.7. Schematic patterns representing basic structural units of lyotropic mesomorphism of amphiphlic lipids. (a) Monolayer at the air/water interface (air can be replaced by oil). (b) Spherical micelle. (c) Flat bilayer in water (a unit of a lamellar phase). (d) Closed bilayer (giant vesicle). (e) Direct cylindrical micelle (a unit of the direct hexagonal phase: oil in water). (f) Inverse cylindrical micelle (a unit of the inverse hexagonal phase: water in oil). (g) Vesicle (small spherical liposome obtained by sonication of a lamellar phase (from Brown & Wolken, 1979, with permission from the authors and the Publisher).

conformations in the monolayer. Albrecht *et al.* (1978) developed this idea further, introducing separate stretching vectors for the hydrophobic region (J_h) and for the polar region (J_p) of the monolayer. Anisotropic fluid and crystalline phases with normal or tilted J_h could then be distinguished.

The search for correspondence between monolayer mesophases and thermotropic smectic liquid crystals was further carried out by Peterson and co-workers (Bibo *et al.*, 1991). Up to 8 known smectic types of molecular order were identified on π-T (two-dimensional pressure-temperature) phase diagrams of fatty acids and esters and the existing smectic nomenclature (Gray & Goodby, 1984; Pershan, 1988) was adopted to designate them. A three-parameter Landau theory of phase transition in monolayers was developed (Kaganer & Loginov, 1993, 1995), involving collective tilt of the molecules (Kaganer *et al.*, 1995).

Considerable interest was raised recently by the phase diagrams of mixed lipid monolayers and domain formation phenomena (Albrecht *et al.*, 1981; Lösche & Möhwald, 1984; Helm *et al.*, 1987), by monomeric and polymeric lipid mixtures (Frey *et al.*, 1987) and by the new effects in lipid-protein monolayers (Panaiotov, 1985).

2.2.2. Phase Diagrams. Polymorphism of Binary Systems

Physico-chemical descriptions of lyotropic polymorphism usually start from very diluted amphiphile solutions and follow the structuring of these solutions at rising amphiphile concentration. Amphiphile-water solutions are monomeric at very low concentration only. The first structuring of the solution, the formation of molecular aggregates, "micelles" (*vid.* Fig. 2.7.b and Fig. 2.13.), at the so-called "critical micellar concentration" (CMC) is well understood, both experimentally and theoretically (Tanford, 1973; Wennerström & Lindman, 1979; Lindman & Wennerström, 1980; Israelachvili, 1992). Thermodynamic reasons for micelle formation are also well rationalized based on the notion of hydrophobic effect and the so-called "principle of opposing forces", *viz.*, the equilibration of the electrostatic repulsion of polar heads and attraction of the chains due to the hydrophobic interaction (Tanford, 1973; Israelachvili *et al.*, 1976; Israelachvili, 1992). At further increasing amphiphile concentration, phenomena like the breaking down of spherical micelle symmetry, the formation of disc-like or rod-like micelles (Israelachvili, 1992), the appearance of intermicellar interactions at closer micellar approach and the mutual arrangement of micelles to form ordered liquid crystalline phases are followed step by step. Such an approach to lyotropic polymorphism is developed for single chain amphiphiles, soaps and detergents, in the first place.

Soaps feature markedly higher water solubility, compared to the double chain lipids, and their CMC is high enough ($10^{-3}-10^{-2}$ M for soaps compared to $10^{-10}-10^{-5}$ M for lipids (Israelachvili, 1992).

A *physical* description of lyotropic polymorphism more naturally begins from the opposite end of the concentration axis of the phase diagram, *viz.*, with the addition of water to a solid crystal of an amphiphilic substance. As a rule, amphiphilic molecules build up crystals of a bilayer structure. This is because intermolecular interaction is maximized by molecular contacts following the "alike with alike" principle: hydrophilic with hydrophilic and lypophilic with lipophilic. Electrostatic interactions (ion–ion, ion–dipole, dipole–dipole, dipole–induced dipole) and hydrogen bonds prevail between hydrophilic groups, while dispersion interactions dominate between hydrophobic chains. In those crystals, alkyl chains are in the all-*trans* conformation and are parallel to one another, often tilted with respect to the polar heads' planes. In soap crystals, interdigitated monolayers are often observed: alkyl chains extend between the two monolayers and the bilayer thickness is of the order of one (but not two) molecular lengths. These data are obtained by X-ray analysis (potassium stearate: Lomer, 1952; phosphatidylethanolamine: Hitchcock *et al.*, 1974; phosphatidyl choline: Pearson & Pascher, 1979) and electron diffraction (Dorset, 1976). Collection of lipid crystal structures is made by Marsh (1990).

Anhydrous lipid crystals display sophisticated thermotropic polymorphism (potassium stearate: Gallot & Skoulios, 1962; phosphatidyl choline: Luzzati & Tardieu, 1974; sodium ricinoleate: Narayan *et al.*, 1992, 1994). Dense packing of heads and chains leads to strong intermolecular interactions; therefore, the melting points of these crystals are rather high (lipids ca. 100–200°C; soaps *ca.* 200–300°C). Substances displaying both thermotropic and lyotropic polymorphism are called "amphitropic" (e.g., gemini diammonium surfactants, Fuller *et al.*, 1996; carbohydrates, Borisch *et al.*, 1996, 1997; etc.).

The addition of a small quantity of water decreases packing density drastically. Water molecules penetrate the space between the polar heads of the adjacent bilayers and this enables them to slide upon each other (Fig. 2.9.). Water decreases the Coulomb attraction between oppositely charged groups of polar heads (e.g., of zwitterionic lipids). As the amount of water further increases, the polar heads of ionic lipids get ionized; a number of hydrogen bonds between the heads start to break in favour of some new hydrogen bonds between heads and water molecules. The resulting loose packing brings the chain melting temperature T_c sharply down; depending on the chain length and degree of unsaturation it could fall even below room

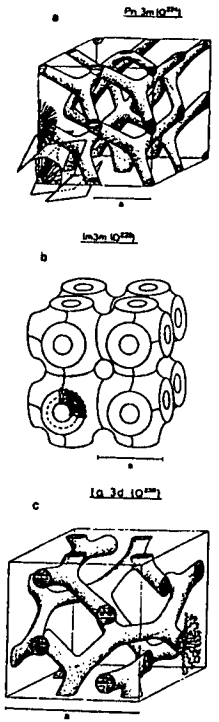

Figure 2.19. The structure and symmetry of the well-established inverse bicontinuous cubic phases. (a) The double diamond cubic phase, space group Pn3m (Q^{224}). (b) The "plumber's nightmare" cubic phase, space group Im3m (Q^{229}). (c) The gyroid cubic phase, space group Ia3d (Q^{230}). (From Seddon, 1990, with permission from the author and the Publisher).

"inverse micellar cubic phase" of space group Fd3m has been found in various ternary systems involving binary lipid mixtures: fatty acid/soap/water, fatty acid/monoglyceride/water, etc. (Mariani *et al.*, 1988; Seddon, 1990b; Seddon *et al.*, 1990). The structure of this Fd3m cubic phase has been solved by low-resolution crystallography by Luzzati

THE COLLOIDAL DOMAIN

Where Physics, Chemistry, Biology, and Technology Meet

Second Edition

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and
Håkan Wennerström

WILEY-VCH

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- The Gordon parameter ($\gamma/V^{1/3}$ N/m^2, where γ and V are the solvent's surface tension and molar volume) provides a useful measure of solvent cohesive energy, as illustrated in Figure 1.11.
- Micelle formation is observed in polar (water, hydrazine, and ethylene glycol, $1.3 < \gamma/V^{1/3}$) and in nonpolar (oil and fluorocarbons, $\gamma/V^{1/3} < 0.3$) solvents, but not in intermediate solvents (alcohols, esters, and amides).

1

Most colloidal phenomena involve a liquid phase as at least one of the actors. In this liquid we can typically identify a *solvent* and one or more *solutes*. Molecular interactions involving colloidal entities, solutes, and solvents determine the properties of the system.

Throughout this book we try to maintain this molecular perspective on the colloidal phenomena. In this chapter we apply this strategy to a description of the general characteristics of amphiphilic assemblies. As we shall see, these colloidal self-assembled structures arise from a delicate interplay between solute–solvent and solute–solute interactions. The term **amphiphilic** indicates that one part of the molecule likes the solvent, while the other does not.

1.1 Amphiphilic Self-Assembly Processes Are Spontaneous, Are Characterized by Start–Stop Features, and Produce Aggregates with Well-Defined Properties

Amphiphilic molecules spontaneously self-organize into a variety of structures. The simplest and best understood of these is the micelle. To characterize the amphiphilic aggregation process, we can begin by considering how adding surfactant to water leads to the formation of this typical structure.

One example of a dual-character molecule possessing the well-defined polar head and nonpolar tail needed to produce amphiphilic behavior is sodium dodecyl sulfate (SDS), whose structure is given in Table 1.1. In aqueous solutions up to 8×10^{-3} M, most of the properties SDS displays are similar to those we can observe for a typical electrolyte such as NaCl. Figure 1.1 illustrates an exception: decreasing surface tension, denoted as γ.

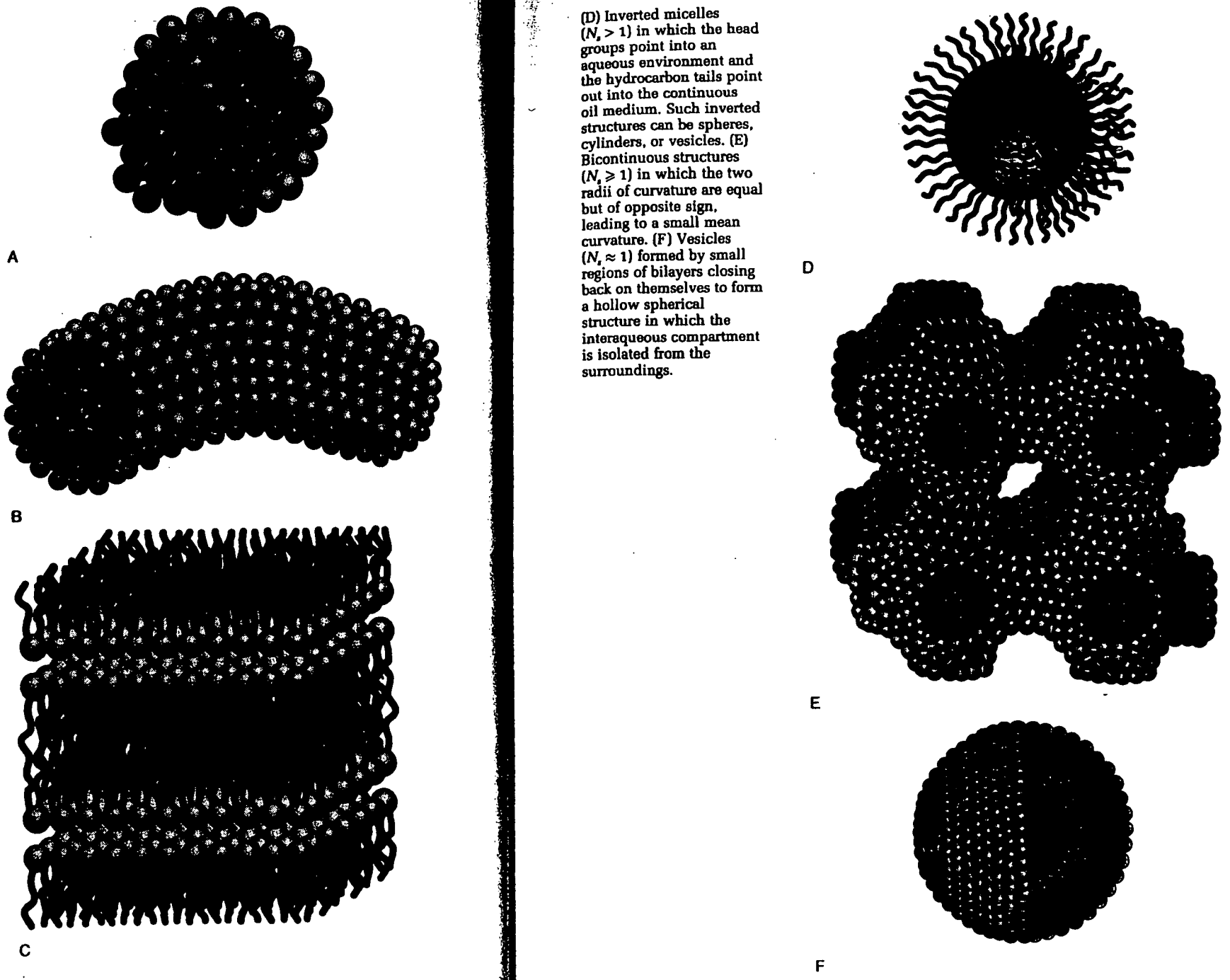


Figure 1.6
Amphiphilic aggregate structures. (A) Spherical micelles ($N_s = 0.33$) in which the micelle's radius $R_{micelle} \approx l_{max}$. For SDS micelles $R_{micelle} = 20$Å, the micelle aggregation number N is 60, and the head group area a is 60 Å^2. The actual cross- sectional area of the sulfate head group is 27 Å^2. Thus, 55% of the micelle's surface involves direct contact between hydrocarbon and water. (B) Cylindrical micelles ($N_s = 0.5$) in which $R_{micelle} = l_{max}$. The ends of the cylinder are capped by hemispheres covered by surfactant head groups. Cylindrical micelles are usually polydisperse because the rod can grow to varying lengths by simply incorporating more surfactants into the cylindrical portion of the micelle. (C) Planar bilayers ($N_s = 1$) in which the bilayer thickness is $\approx 1.6 l_{max}$ in the liquid state. Such structures can grow in the bilayer plane without limit and usually curve back on themselves to minimize exposure of hydrocarbons at the edges of the bilayer to water. (D) Inverted micelles ($N_s > 1$) in which the head groups point into an aqueous environment and the hydrocarbon tails point out into the continuous oil medium. Such inverted structures can be spheres, cylinders, or vesicles. (E) Bicontinuous structures ($N_s \geq 1$) in which the two radii of curvature are equal but of opposite sign, leading to a small mean curvature. (F) Vesicles ($N_s \approx 1$) formed by small regions of bilayers closing back on themselves to form a hollow spherical structure in which the interaqueous compartment is isolated from the surroundings.

group. Because amphiphiles with single hydrophobic chains have surfactant parameters of less than 0.5, they are constrained—at least in dilute solution—to form micellar aggregates. Adding a second hydrocarbon chain doubles v, while a_0 and l ramain essentially the same. In effect, the addition doubles v/a_0l into the range 0.5–1. Hence, double-chain surfactants tend to form bilayer structures such as vesicles and lamellar liquid crystals, whose curvatures are inherently lower than those formed by their single-chain counterparts.

A comparable, although quantitatively different, approach to analyzing aggregate structures used the curvature concept explicitly. In this approach, the crucial parameter is not the surfactant number N_s but the preferred mean curvature of a surfactant film. We can define the **mean curvature** H at a point on a surface as

$$H = \tfrac{1}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \qquad (1.3.8)$$

where R_1 and R_2 are the radii of curvature in two perpendicular directions, as illustrated in Figure 1.7. For a sphere, $R_1 = R_2 = R$ and $H = 1/R$, for a cylinder, $R_1 = R$, $R_2 = \infty$ and $H = 1/2R$, while for a planar bilayer, $H = 0$. A value of $H = 0$ also can occur on a saddle-shaped surface in which $R_1 = -R_2$. To assign a sign to the radii of curvature, we must define a normal direction, n, for the surface. By convention, $\vec{n}$ is usually positive, pointing toward the polar region, and therefore the curvature of inverted aggregates is negative.

We will consider the specific properties of the various amphiphilic aggregates shown in Figure 1.6 in later chapters. The rest of this chapter deals with the general features of solution and solvents.

1.4 Understanding the Origin of Entropy and Enthalpy of Mixing Provides Useful Molecular Insight into Many Colloidal Phenomena

Throughout this book we will be concerned with the thermodynamics of the self-assembly of surfactant molecules into aggregates, the properties of polymer solutions, and the behavior of colloidal entities such as emulsions. To obtain a basis for these more complex systems we start by considering the mixing of two pure liquids. Figure 1.8 shows three possible outcomes of mixing two components:

1. Formation of a homogeneous solution resulting from complete miscibility at all compositions. An example is t-BuOH and water at 25°C.
2. Formation of two phases containing different concentrations of the two components. An example is n-BuOH and water at 25°C.

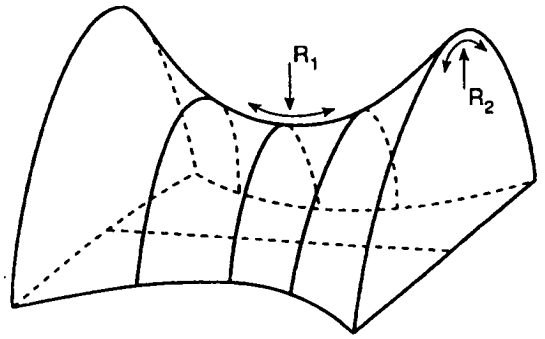


Figure 1.7
Radius of curvature. For a surface in three dimensions, two mutually perpendicular radii of curvature, R_1 and R_2, can be specified at each point. On a saddle-shaped surface, the two radii of curvature have opposite sign. Here R_1 and R_2 are shown at two different points on the surface.

3. Retention of two unmixed phases, illustrated by water and mercury.

In a thermodynamic description, the outcome of these mixing processes differs because the free energies of mixing, ΔG_{mix}, are different. At constant T and P, the Gibbs free energy is

$$\Delta G = \Delta H - T\Delta S \qquad (1.4.1a)$$

and for a mixing process

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} \qquad (1.4.1b)$$

where $\Delta G_{mix} = G_{final\ solution} - G_{initial\ solution}$. We can define ΔH_{mix}

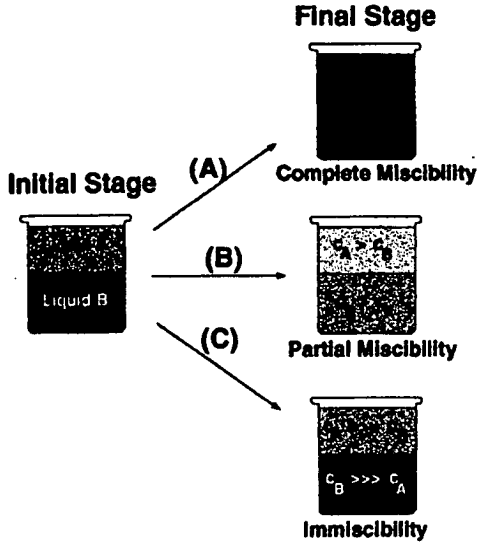


Figure 1.8
Three possible outcomes of mixing equal amounts of two liquids A and B: (a) Formation of a homogeneous mixture; (b) Formation of two phases, one solution of B in A and one solution of A in B; (c) retention of the two unmixed phases with the second component present in small amounts.

quantity determined by the length of the hydrocarbon chains while there is no molecular limit to bilayer growth in the lateral direction. In an ideal situation, bilayers attempt to minimize the degree to which the hydrocarbons at their edges are exposed to the aqueous environment by extending indefinitely in the lateral direction. In reality, finite samples show defects, and bilayers tend to close up on themseves, leading to a variety of macroscopic structures described in the next section.

In micellar solutions, the relaxation times for monomer exchange range from 10^{-3} to $10^{-6}\,s^{-1}$, and they involve a diffusion-limited process that depends directly on the monomer concentration. In bilayers, monomer exchange is considerably slower, as predicted by their much lower monomer solubility.

Lifetimes for typical micelles vary from 10^{-3} to 10^{-1} s. With bilayers, such transformations involve rearranging the entire bilayer structure, and a particular specific macroscopic state often reflects the methods used to prepare the sample. Thus, micelles respond rapidly to changes in their environment, while bilayers are much more sluggish. It can require hours, days, or even years for a bilayer to achieve an equilibrium state.

The transport properties of micelles and bilayers also display major differences. With spherical micelles, all directions are equivalent, and a surfactant molecule can diffuse very rapidly within a micelle. A bilayer, on the other hand, has two distinct diffusion modes. The first involves lateral diffusion within the bilayer plane. It occurs rapidly since it mainly involves the amphiphile chains moving through the oillike region of the bilayer. The second diffusional mode is slow since it involves moving the head group from one side of the bilayer to the other. This "flip-flop" requires the polar head group to rotate through the oillike interior of the bilayer. In pure bilayers, the time required to execute this flip-flop process is typically hours to days.

6.1.4 Pure Amphiphiles Form a Range of Bulk Bilayer Phases

The lamellar liquid crystal is the generic bulk phase of a bilayer-forming amphiphile. As illustrated in Figure 1.6c, a lamellar liquid crystal consists of a stack of bilayers separated by aqueous films. In the liquid crystalline state, the molecules diffuse freely in the lateral direction, while they move in the perpendicular direction only on a very long time scale. The apolar chains behave as in a liquid with a sizable fraction of gauche conformations. The forces acting between bilayers determine the thickness of the aqueous layer. As discussed in the previous chapter, a range of forces is operating, and bilayer systems are very useful in the study of these forces.

When a lamellar phase is cooled, it undergoes a phase change that can lead to the formation of a crystal or sometimes to a stable or metastable gel phase. Figure 6.3 shows some typical gel phase structures. Their characteristic feature is that the alkyl chains are crystallized while liquidlike solvent still

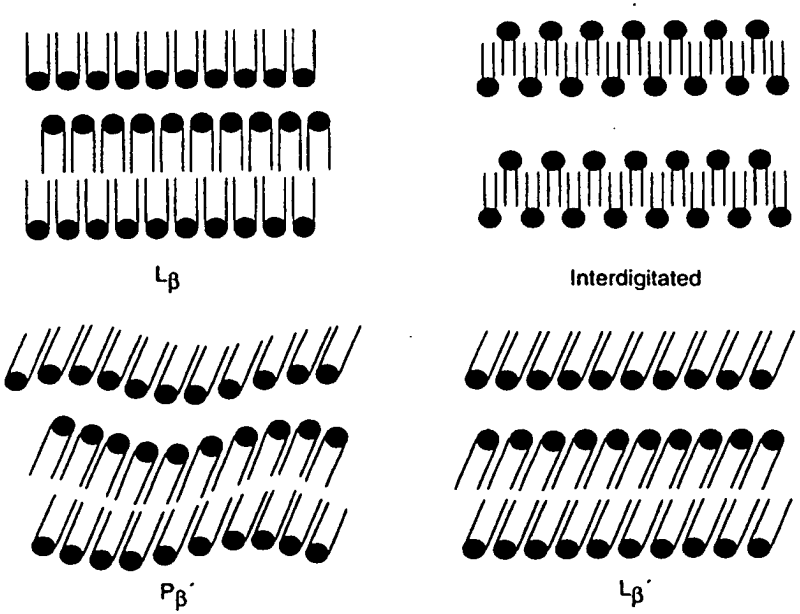


Figure 6.3
When alkyl chains crystallize, they have a cross-sectional area of $\approx 20\,Å^2$ per chain in a plane perpendicular to the direction of the chain. If the area of the polar groups in the bilayer matches that of the chain(s), a structure $L_{\beta'}$ is adopted. In the more likely case of a mismatch between polar head and chain areas, a tilted structure, as in $L_{\beta'}$, or a rippled structure, as in $P_{\beta'}$, can appear. For single-chain amphiphiles, chains may even interdigitate, making the thickness of the apolar layer the same as the length of a single chain.

exists between the bilayers. The crystalline state of the chains implies that more stringent conditions are imposed on the packing. In this case, a mismatch can easily arise between the cross-sectional area per chain and the head group area. To accommodate such a mismatch, the chains may tilt relative to the bilayer plane, the bilayer may develop a ripple, or the chains may even interdigitate, depending on the degree of mismatch.

Bilayers form the basic structural element in other ordered phases. Figure 6.4 shows a representative bicontinuous phase of cubic symmetry in which the bilayer is draped on a surface of zero mean curvature. Because the structure is bicontinuous, the amphiphile and the solvent each diffuse in three dimensions within its own medium, displaying an effective diffusion coefficient only slightly smaller ($\sim 2/3$) than its value in a homogeneous solution. Under these circumstances, the bilayer separates the solvent into two distinct regions. Molecular transport from one region to the other involves passage through the bilayer, a fact that has particular relevance for biological membrane systems.

In isotropic solutions, bilayers can appear in the form of vesicles shaped like spherical shells (see Figure 1f). Depending on the nature of these vesicles, we usually distinguish between small unilamellar vesicles (SUVs; radius $\lesssim 100$ nm), large unilamellar vesicles (LUVs), and multilamellar liposomes. There is no sharp distinction between liposomes and a lamellar phase with excess solvent. Vesicles tend to occur most readily for systems with surfactant numbers N_s slightly below one. They are seldom true equilibrium systems, but they are often meta-

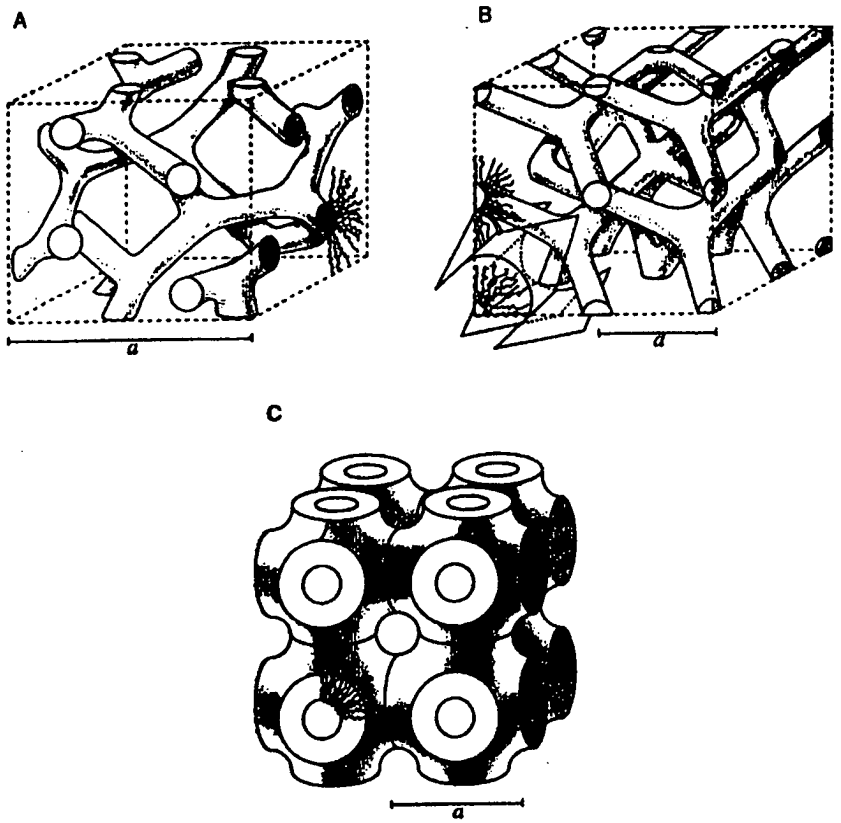


Figure 6.4
Illustration of three different arrangements of bilayers in cubic symmetry: (a) a gyroid structure; (b) a double diamond (as in two interwoven diamond lattices); and (c) plumbers nightmare with the same symmetry as in the monolayer version of Figure 1.6e. (Seddon, *Biochem. Biophys. Acta* **1031**, 1–69 (1990).)

stable enough to allow detailed physical and chemical studies that have proved very valuable. In vitro studies of membrane proteins are one example.

An intriguing bilayer arrangement commonly found in surfactant systems is the so-called sponge or L_3 phase. In this phase, the bilayer extends for an unlimited distance in three dimensions, forming a single macroscopic aggregate with isotropic properties. This phase corresponds to a disordered version of a cubic phase shown in Figure 1.6e and it occurs for surfactant numbers N_s slightly larger than one.

The difference in free energy between the various bilayer structures often is merely on the order of a small fraction of kT per molecule. Thus, small changes in temperature or composition can cause the transformation from one structure to another.

6.1.5 Vesicles Can be Formed by Several Methods

Vesicles commonly occur in the living cell and many physiological processes involve steps where vesicles are formed or

consumed by fusion with another bilayer system. The importance of vesicles is not restricted to living systems and they are also very useful for in vitro studies of membrane physical and chemical properties. Because most vesicles are thermodynamically unstable, the properties of a vesicle solution depend on how it is prepared.

A simple but nonselective way of producing vesicles is to subject a lamellar dispersion to ultrasound, such as a lamellar phase containing excess water. The sound waves tear the lamellae, which then can reseal into vesicles. The smaller the vesicle, the less the perturbation affects it; so the result of the ultrasound treatment is a broad but skewed size distribution of vesicles that peaks at rather small aggregates, typically $R \simeq 150$—200 Å. A disadvantage of the sonication process is that the large energy input may chemically degrade the lipid.

Cholate dialysis represents a more controlled method for preparing vesicles. In this technique we prepare a dilute solution of lipid–cholate mixed micelles and dialyze it. High monomer solubility enables the cholate to readily pass the dialysis membrane. As a result, the cholate content of the lipid solution will decrease slowly but steadily, and the lipid content of the micelles will increase until the large aggregates finally close to form vesicles. These vesicles possess a more uniform size, which we can control by varying parameters such as salt content, temperature, and pH.

Another way of producing vesicles is to inject a solution of the lipid in an organic solvent or solvent mixture into an excess of water. Large unilamellar vesicles form as the organic solvent evaporates or is dissolved in the water. Because the experimental conditions can vary greatly, obtaining vesicles of specified properties with this method is to some extent an art rather than a science.

In many instances, vesicles appear "spontaneously" as we prepare the lipid–water system. We can obtain large unilamellar vesicles by simply taking a dry lipid powder and adding the aqueous solvent. With charged lipids, continuous dilution of the system can lead to the formation of vesicles under the action of double-layer repulsions.

The kinetic stability of a vesicle system depends on the rate of the process by which two vesicles fuse to form a larger one. Vesicle stability is really an example of the general problem of colloidal stability, discussed further in Chapter 8.

The fusion process is easier to understand if we think of it as occurring in two steps. In the first step, the respective bilayers come into proximity. The occurrence of this step depends strongly on interbilayer forces. For charged systems with low electrolyte concentrations, double-layer repulsion largely prevents two vesicles from meeting. For zwitterionic phospholipid vesicles, a sizable shortrange stabilizing force also tends to prevent close molecular contact.

The second step in the process actually fuses the two bilayers. Any substantial barrier to this process may cause the two vesicles to aggregate and stay together for long periods without ever fusing. Factors that influence the fusion process

surface tension—diverge/vanish as the independent variable, for example T, approaches a critical value, T_c.

The transformation of a gas–liquid two-phase system to a supercritical fluid at the critical point provides an example. As the physical differences between the two phases diminish and merge at T_c, long range molecular correlations become important.

Continuous transformations can be characterized by power law expressions with characteristic exponents (eqs. 10.4.1–10.4.4) that can be predicted from statistical mechanics. Surprisingly, the same set of critical exponents describes many seemingly diverse physical systems.

We can illustrate many features of continuous phase transitions using regular solution theory. The critical exponents are inaccurate, reflecting the failure of mean field theory to correctly account for long range molecular correlations.

Other Sources of Continuous Transformations

So far we have focused on macroscopic systems possessing a few well-defined components. Other sources of apparently continuous phase transformations include:

- Systems in which finite size effects play a dominant role, such as in the formation of small micelles leading to a broad CMC.
- Systems containing impurities or inhomogeneities, which broaden phase transformations.
- Systems in which equilibrium times are long compared to observation times, as in glass transitions for polymers.

10

In colloidal systems, interparticle or interaggregate interactions can easily be strong enough to promote macroscopic ordering that can lead to the formation of a sequence of phases. Slight variations in conditions may be enough to trigger phase changes that in turn induce major changes in macroscopic properties. Although surfactants and biological lipids display particularly rich behavior, the phenomenon is also important in other colloidal systems.

The study of phase equilibria fulfills a dual purpose. As the most important factor in the thermodynamic characterization of a system, phase behavior often provides an essential clue to macroscopic behavior. In addition, we can link phase behavior in an intimate but nontrivial way with molecular or particle interactions. If we understand this connection properly, we can control phase behavior by changing interactions on a molecular level. Conversely, we can study interaggregate interactions by observing phase behavior.

10.1 Phase Diagrams Depicting Colloidal Systems Are Generally Richer Than Those for Molecular Systems

10.1.1 Several Uncommon Aggregation States Appear in Colloidal Systems

Colloidal systems show a greater diversity in aggregation states than we find in molecular systems. Reviewing the general terminology used to describe different states and phases can help us appreciate this fact, but we must remember that whenever we attempt to systematize a complex physical reality, we encounter borderline cases that may not fit a given classification system.

At ambient temperatures, matter, exists in a solid, liquid, or gaseous state or in a mixture of these. Dynamic properties at the molecular level provide the basis for characterizing aggregation states. In a **solid**, a molecule's translational motion is slow to very slow. In a **liquid**, molecules (or colloidal particles) diffuse quite rapidly in space and interact constantly with their neighbors. In a **gas**, a molecule may travel unperturbed over a distance relatively large compared to its size before colliding with another molecule.

These three main aggregation states display a range of phase types, each with its own specific characteristics. For example, the most common form of solid is a **crystal**, in which molecules are arranged with long range positional and orientational order. Closer to the melting point, a crystal can undergo a phase transition, becoming noticeably softer. It maintains full positional order in this **plastic crystal** state, but it loses some orientational order and molecules rotate in their lattice sites. This behavior commonly occurs for compounds with long hydrocarbon chains.

In **amorphous solids**, molecules (or atoms) are not located in precisely determined lattice sites, as they are in crystals. An important example—found commonly in solid polymers but also seen in other colloidal systems—is a **glass**. The molecular arrangements we observe in glasses are very similar to those found in liquids, while the local dynamic properties are typical of solids. Amorphous solids often represent nonequilibrium structures, but because of the slow dynamics in the solid state, they can remain unchanged for indefinite times.

Although molecules diffuse rapidly in a liquid, in principle, a long range order can be maintained. However, for ordinary **isotropic liquids** only a short range order exists between neighboring molecules, and no correlations exist between molecules farther away. Most substances melt from a crystalline solid to an isotropic liquid. This change implies a transition from a very ordered state to a totally disordered one.

However, a number of compounds show a more gradual melting behavior, with one or more transitions between the crystalline solid and the isotropic liquid. These intermediate phases or **liquid crystals**, retain some, but not all, of the macroscopic order present in crystal, while there is a high molecular mobility.

Liquid crystalline phases that are induced by temperature changes are denoted as thermotropic liquid crystals. There is a considerable chemical diversity among the substances forming thermotropic liquid crystals. The most well known type is the one containing a stiff elongated aromatic backbone with a short alkyl chain at the ends. However, compounds like cholesterol esters, pure soaps, and some polymers can also form liquid crystals. In colloidal systems we are normally dealing with two or multicomponent systems, with particles or aggregates in a solvent. In this case, one uses the term lyotropic liquid crystals. The thermotropic and lyotropic liquid crystals differ somewhat in the chemical interactions causing the formation of the ordered phases, but the thermodynamic properties are basically the same.

In a **smectic** liquid crystal, molecules show long range (macroscopic) orientational order and also long range translational order in one or two directions. The term **smectic**, which is derived from the Greek word for soap, sometimes refers specifically to a layered structure like those found in the lamellar liquid crystals discussed in Chapter 6, but other forms of positional ordering are also possible like the hexagonal or cubic phases we have mentioned in Chapter 1. Figure 10.1 illustrates a typical molecular ordering in a thermotropic smectic phase.

When a smectic phase is heated, it can melt into an isotropic liquid. More often, however, it transforms into a phase in which the long range positional order has disappeared, but long range orientational order remains. The nature of this orientational order depends on whether the molecules are optically active (chiral).

A nonchiral molecule produces a **nematic** liquid crystalline phase, as illustrated in Figure 10.2, in which the preferred orientation is homogeneous throughout the system. Because

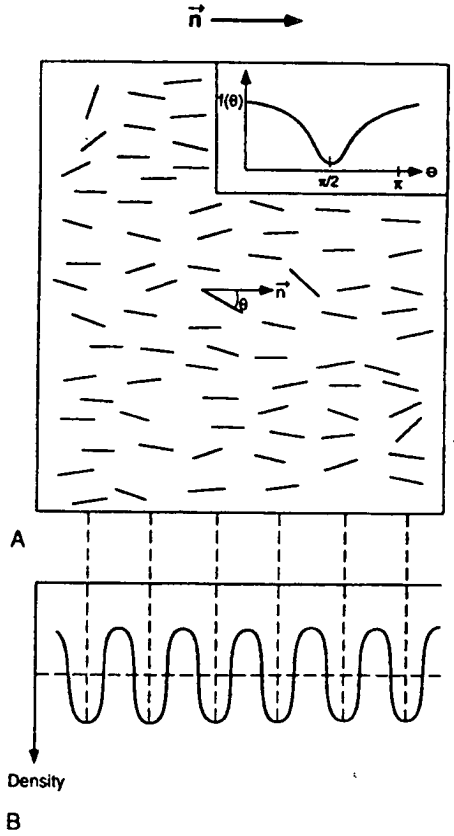


Figure 10.1
Illustration of molecular order in a smectic phase. The molecules are preferentially oriented along the direction n. Acareful examination also reveals a layering in the direction of n. When the layering and the orientation lie along the same direction, n, the phase is called smectic A, otherwise it is called a smectic C phase. (A) The orientational distribution function $f(\theta)$ with maxima at $\theta = 0, \pi$ and a minimum at $\theta = \pi/2$. (B) The center-of-mass density along the director showing a periodic variation defining the layer thickness.

The total amount of matter is constant so that

$$dn_1^\beta = -dn_1^\alpha \quad \text{and} \quad dn_2^\beta = -dn_2^\alpha \tag{10.1.6}$$

Thus,

$$dG = (\mu_1^\alpha - \mu_1^\beta)dn_1^\alpha + (\mu_2^\alpha - \mu_2^\beta)dn_2^\alpha \tag{10.1.7}$$

and at the minimum, $dG = 0$, that can only occur for

$$\mu_1^\alpha = \mu_1^\beta \quad \text{and} \quad \mu_2^\alpha = \mu_2^\beta \tag{10.1.8}$$

because n_1^α and n_2^α can vary independently. In the general case,

$$\mu_i^\alpha(X^\alpha) = \mu^\beta(X^\beta) \tag{10.1.9}$$

for all components i, where X^α and X^β denote the compositions of the two phases α and β, respectively.

By definition

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,p,n_j} \tag{10.1.10}$$

so we can calculate the chemical potential if we know G as a function of the composition. The intercept method provides a convenient way to graphically determine μ_1 and μ_2 for two-component systems. It is particularly useful for determining qualitative features.

Figure 10.4 illustrates the method through a plot of $G/(n_1 + n_2)$ versus X_1 (or X_2). We obtain the chemical potentials

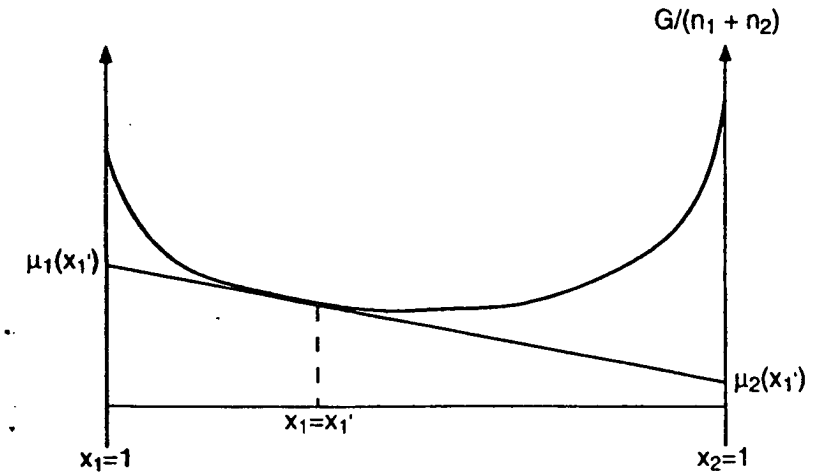


Figure 10.4
The intercept method for determining chemical potentials. Plot free energy G over total amount $(n_1 + n_2)$ versus concentration. The chemical potentials at a composition X_1' are obtained by constructing the tangent to the $G/(n_1 + n_2)$ curve at the particular concentration. The two chemical potentials, $\mu_1(X_1')$ and $\mu_2(X_1')$ are then obtained as the intercepts at $X_1 = 1$ and $X_2 = 1$, respectively.

at an arbitrary composition X_1' by taking the tangent of the curve, $G/(n_1 + n_2)$, at X_1'. The intercepts of this tangent at $X_1 = 1$ and $X_2 = 1$ are the chemical potentials $\mu_1(X_1')$ and $\mu_2(X_1')$. (For proof see any text on physical chemistry.)

With two components, there are three independent intensive variables. At fixed T and p, only one is left. Now the chemical potentials, μ_1, and μ_2, are both intensive. Thus, they cannot be varied independently at constant T and p. The interdependence between the two is expressed in the Gibbs–Duhem equation

$$n_1 d\mu_1 = -n_2 d\mu_2 \tag{10.1.11}$$

If we measure the concentration dependence of the chemical potential of one component, we can calculate the concentration dependence of the other by using eq. 10.1.11. This explains, for example, how we can use a measurement of the osmotic pressure—that is, the chemical potential of the solvent—to study interactions between solute molecules or colloidal particles.

10.1.4 Phase Diagrams Conveniently Represent Phase Equilibria

Phase diagrams provide us with a useful visual way to represent phase behavior. In one diagram we can summarize a large body of experimental data, and we also can use the phase diagram for predictive purposes.

A two-component system may have up to three degrees of freedom, but we can display only two dimensions conveniently in a graphic representation. Pressure usually influences condensed phases only slightly. In such a case, we decrease the number of degrees of freedom by one by holding the pressure constant (e.g., at 1 atm), while we vary temperature T and composition X.

Figure 10.5 illustrates some general features of T–X (p fixed) diagrams through a generic case of two components A and B, which form separate solid phases, yet show miscibility in the liquid state l. For one-phase regions with two degrees of freedom ($f = 2$), T and X vary independently, and the phase remains the same, although it exhibits continuously varying properties.

When two phases are in equilibrium, $f = 1$. In the diagram, these regions also have a two-dimensional character, but changes in total composition do not change the individual composition of the two phases in equilibrium. Their only effect is to change the relative amount of each phase. For example, a system containing one mole at composition X and temperature T (such as the one illustrated in Figure 10.5) will separate a moles of solid A and b moles of solid B in such a way that $a + b = 1$.

Conservation of component 1 implies

$$X_2' = aX_2^A + bX_2^B \rightarrow a(X_2^A - X_2') = b(X_2' - X_2^B) \tag{10.1.12}$$

which is called the **lever rule.**

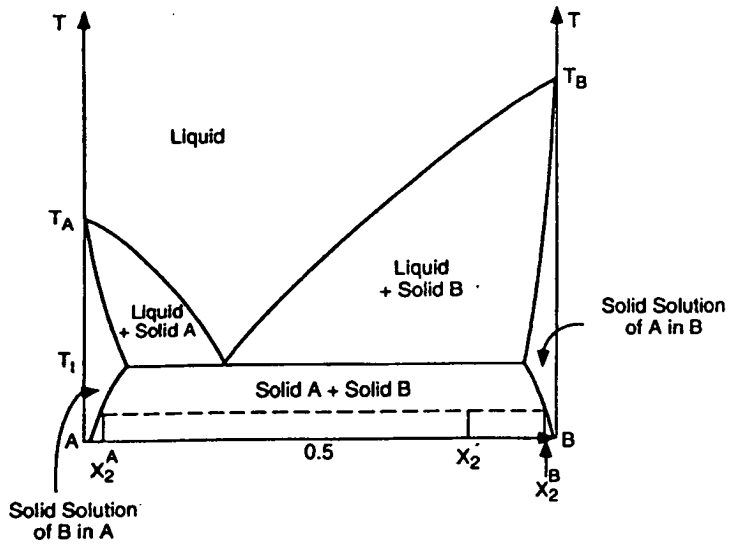


Figure 10.5
Binary T–X phase diagram showing one-phase and two-phase areas and a three-phase line at $T \simeq T_i$. Use of the lever rule in a two-phase area is also shown. The diagram represents the system Ag(A)–Cu(*B*).

The horizontal straight line at temperature T_i is called a three-phase line. The three phases, *A*, *B*, and *l*, are in equilibrium at this temperature, and consequently $f = 0$. No degree of freedom exists, and both the temperature and the compositions of the phase in equilibrium are unique.

Systems with three components may have as many as four degrees of freedom. To represent the phase behavior of such a system in a two-dimensional diagram, we must fix two degrees of freedom, usually T and p. Then we can represent the composition in a triangular diagram. As an example, Figure 10.6 shows the phase equilibria for a water–potassium decanoate ($C_9H_{19}COOK$)–octanol system.

Figure 10.7 demonstrates how to represent the composition by means of such a diagram. Each corner represents a pure component, while the opposite base of the triangle represents the binary system of the other two components. We arrive at the relative amount of component *A* at an arbitrary point *P* by drawing a line parallel to the *A* base. As shown in Figure 10.7, the intersection of the parallel line with the sides of the triangle determines the fraction of *A*.

The phase diagram divides the triangle into three types of area. One-phase areas have two degrees of freedom and can take any shape. Two-phase areas may have four corners joined by two opposing straight lines and two opposing curved lines, or we can replace the straight line(s) by concave lines ending in a critical point. These phases have only one degree of freedom, which shows that variations in one direction do not change intensive variables. In a complete phase diagram, we mark the

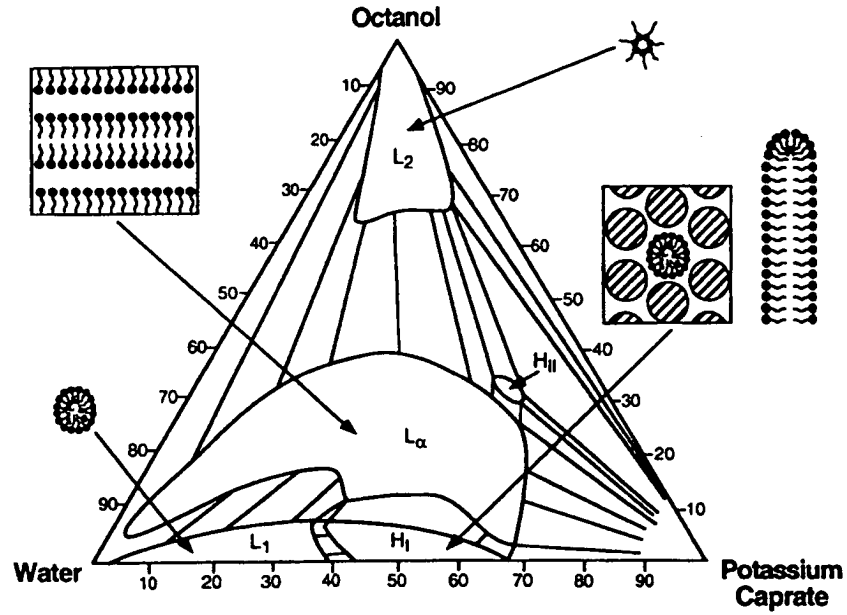


Figure 10.6
Phase diagram of the ternary system water–potassium decanoate (captrate)–octanol at constant pressure (1 atm.) and constant temperature (20°C). L_1 indicates a micellar and L_2 a reversed micellar solution. H_I is a normal hexagonal and H_{II} a reversed hexagonal liquid crystalline phase; L_α is a lamellar phase. (B. Jönsson and H. Wennerström, *J. Phys. Chem.* **91**, 338 (1987).)

invariant direction by so-called tie lines, which connect points on the two opposing curved lines as exemplified in Figure 10.6. Tie lines connect one-phase points in equilibrium. Only the proportions of the two phases change for points along the same tie line, and the lever rule (eq. 10.1.12) applies.

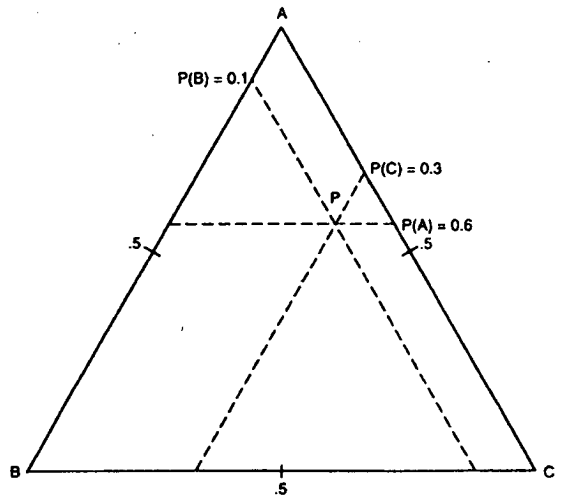
Finally, for three phases in equilibrium, $f = 0$. This situation occurs in so-called three-phase triangles. For a point within such a triangle the three phases represented by the corners of the triangles coexist in equilibrium. The relative amounts are determined in the same way as explained in connection with Figure 10.7, except that we are no longer dealing with an equilateral triangle.

10.1.5 Determining Phase Equilibria Is a Demanding Task

Determining the phase equilibria or phase diagram is usually the most important characterization of the macroscopic properties of a colloidal system. In general, determination of a complete phase diagram involves a lot of work. The basic principle is to mix the components and observe the number and nature of the phases. However, this can be done in a number of ways, depending on the particular characteristics of the sample.

The most obvious, direct, and often the most versatile method, is direct visual observation, be it by the naked eye or

Figure 10.7
Evaluating the composition at a point P in a triangular diagram. The relative amounts of A, B, and C are 0.6, 0.1, and 0.3, respectively.



in a polarizing microscope. Isotropic liquid phases typically phase separate readily, and mixing at a given composition and analyzing the amount and composition of the different phases in equilibrium can make it possible to determine a tie line. So when phases readily separate macroscopically, determining the phase diagram is a straightforward task.

However, colloidal systems that abound in liquid crystals and other viscous phases require a long time for achieving a macroscopic separation of the sample into two distinct regions with a single planar interface. It is more common that phases in equilibrium are dispersed in one another with domain sizes of tens or hundreds of micrometers. For each prepared sample, we face three questions:

How many phases are present?

What is the nature of the phases (liquid, liquid crystal, or solid) and what is the symmetry?

What are the compositions of the phases (if more than one phase is present)?

If macroscopic phase separation does not exist, we are forced to perform measurements on the whole sample and obtain some sort of macroscopic average. The three most common approaches are calorimetry, (x-ray) scattering, and spectroscopic (NMR, ESR, fluorescence) measurements.

Calorimetric measurements are useful only for making a T–X diagram. Differential scanning calorimetry (DSC) readily measures the heat capacity as a function of the temperature, T. By repeating the measurement for a range of compositions, we can localize the temperatures of three-phase lines and of

melting transitions. (See, e.g., Figure 6.18). However, phase changes that involve only small changes in enthalpic properties may remain undetected.

Small-angle x-ray or neutron scattering is sensitive to the structure of the phase. Thus, it is very useful for determining the nature of a phase, although it is more difficult, but still possible, to use the method when several phases are present.

Spectroscopic methods rely on the fact that spectroscopic properties are often sensitive to the local molecular environment. Thus, the same molecule in different phases will give different spectroscopic responses. In a multiphase sample, this behavior can be very useful, making it possible to count the number of phases in a straightforward way. Figure 10.8 shows as an example a series of deuterium NMR spectra used to construct the phase diagram of the system dipalmitoylphosphatidylcholine–water shown later (in Figure 10.21). By measuring the number of signals, their intensity, and the magnitude of the quadruple splittings, the combined information more than suffices to construct the phase diagram.

We conclude this section by noting that some occasions require a rapid method for determining phase behavior. It is no coincidence that such methods have been developed at the scientific laboratories of the two leading detergent producers in the world, Procter & Gamble and Unilever. A common principle is to take a surfactant crystal, place it in a microscope between coverslips, and let water penetrate into the crystal from one side by a diffusion process. During the diffusion process, a concentration gradient is generated and, in principle, the whole compositional range can be sampled across the coverslip. By focusing the (polarizing) microscope at different positions along the concentration gradient, one can determine the sequence of phases. Phase boundaries appear as sharp lines, making it relatively easy to detect a change in phase structure.

10.2 Examples Illustrate the Importance of Phase Equilibria for Colloidal Systems

A phase transition or an equilibrium between two phases typically occurs when a balance exists between two macroscopic states: one more ordered (with a low energy and entropy) and one less ordered (with a higher energy and entropy). In colloidal systems, interactions between particles can easily be of order kT and higher, yet entropies of mixing N_A and N_B objects are the same irrespective of the size of the objects. Consequently, ordered phases occur more readily in colloidal than in small molecule systems. This observation is particularly true for self-assembling systems whose intricate interplay between inter- and intra-aggregate interactions results in a particularly rich phase behavior. We will discuss a number of colloidal systems in which phase behavior constitutes an important aspect of the properties of the system in general.

Phase Transitions in Liquid Crystals

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Chapter 26

INTRODUCTION AND GENERAL THEORY OF LYOTROPIC LIQUID CRYSTALS

D. ROUX

1. Introduction

Amphilic molecules incorporate two antagonistic chemical functions. One part of the molecule is very soluble in water (hydrophilic) and another part is very soluble in an organic solvent (hydrophobic). Due to these opposite effects the molecules in solution aggregate spontaneously into objects of very different size and shape, among which one principally observes spheres, cylinders and twodimensional objects (membranes). Many of the phases observed in a typical phase diagram are liquid crystals. For example, the lamellar phase, which consists of a stack of membranes with a long range order in the direction perpendicular to the plane, is a smectic A phase. At first these phases appeared relatively uninteresting, but now they have been extensively studied and they exhibit extremely interesting and peculiar behavior. In certain cases, close to a transition toward nematic or hexagonal phases, the lamellar phase cannot be described as a stack of flat membranes but exhibits many defects that can be observed experimentally.In principle, the lyotropic nature of the phase allows the repeating distance d between the membranes to vary. In many systems this variation remains small (typically ranging from 30 to 60 Å), however in certain cases it is possible to prepare lamellar phases with extremely large repeating distances (up to several 1000 Å). These systems are especially interesting because, as we will see, they permit us to study the interactions between membranes over a wide range of conditions. They are also a unique example of a colloidal smectic A phase.

The reason that extreme dilutions may exist is due to the existence of long range repulsive interactions between membranes. Two cases have been identified and studied either the interaction is electrostatic in origin, or it is due to the enhancement of thermally excited undulations of the membranes.

The description of this smectic phase can be carried out at two levels (corresponding to two length scales) without the necessity for a complete microscopic theory. For lengths larger than the repeating distance d the system behaves as a regular smectic phase, but for smaller lengths it is still possible to have a continuous description (at least for dilute phases). This description corresponds to a colloidal system and can be done in terms of interacting membranes. Consequently, it is possible to use a simple theory to link the macroscopic properties of the phase such as the elastic constants (compressibility B and bending constant K_1), to the microscopic

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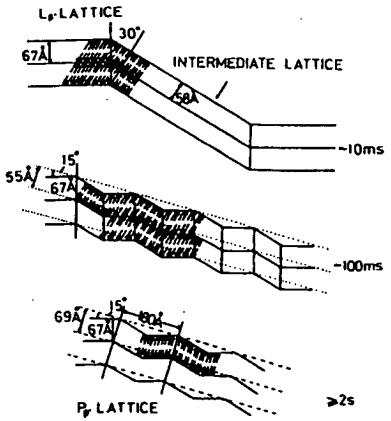

Fig.8. Schematic model of the mechanism of the $L\beta' \rightarrow P\beta'$ phase transition in DPPC, involving the rapid formation of an intermediate 58 Å lattice originating from the parent $L\beta'$ lattice through disclination by an angle of 30°. (From Ref. 4).

3. Polymorphism of Lipids: Non-Lamellar Phases

3.1. The Structure of Cubic Phases

The many studies that have been performed on lipid extracts, purified lipid components and synthetic lipids have established that, under suitable conditions of temperature and hydration, lipids may spontaneously form lamellar phases[7,22]. As reported above, such phases consist of one-dimensional periodic stacks of identical bilayers, each separated by a water layer of constant thickness. However, it has been shown that this lamellar organization is only one of the large variety of liquid crystalline structures which may be adopted by hydrated lipid systems[15,22,23]; to date, among non-lamellar lyotropic arrangements, the cubic phases appear as the most complex and "intriguing"[23,24].

Non-lamellar phases can be described by using a very pictorial type of criterion[6,25]. In fact, considering the molecular structure of an amphiphilic molecule, two different moieties can be visualized in all lyotropic phases, i.e., the regions occupied by the hydrocarbon chains and those occupied by the water, the interface being covered by the polar groups of the molecules. As general rule, it appears possible to identify the shape of the polar (or paraffinic) regions and then to describe with simple geometrical considerations such "structure elements"[6,15,25]. Therefore, the whole structure can be described by considering that the hydrocarbon or the polar medium forms a continuous matrix into which structure elements of opposite polarity are fitted. Moreover, it must be also considered that in the lyotropic phases, and with the exception of those formed by infinite sheets (namely the lamellar phases), a topological distinction could be made between the interior and the exterior volumes of the structure elements[15,22]. In particular, when the paraffinic chains fill the volume inside the structure element and the water the outside region, the structure is called of type I (oil-in-water, normal). On the contrary, the structure is type II (water-in-oil, inverted) when the structure element appears to be filled by the water and embedded in a paraffinic matrix (see, for example, Fig. 9).

It must be also noticed that, with the exclusion of the two-dimensional Pδ phase, in such phases the hydrocarbon chains show a liquid-like organization[6,26] , i.e. the so-called α conformation. This conformation can be visualized as highly disordered, like that of a liquid paraffin, even if, as a consequence of the fact that one chain end is anchored at the interface, the average chain orientation is perpendicular to the lipid-water interface[26]; this orientation is more and more pronounced as the area-per-chain (S_{ch}) decreases (when the water content decreases, for example). It must be also noticed that such a "liquid" may well display complex molecular movements[26].

Moreover, in the non-lamellar phases, simple geometric considerations indicate that the chains must be folded in fairly regular way in order to uniformly fill the oddly shaped volumes offered to them[22,26,27]. However, the conformation of the chains does not appear to be profoundly different in the lamellar and the non-lamellar phases, as assumed or proposed by some authors[22,26,27].

Regarding the structure of non-lamellar phases, we will limit ourselves to the description of the most prevalent hexagonal and cubic phases. In the hexagonal H phase (sketched in Fig. 9), the structure elements are rigid infinitely long rods, all identical and crystallographically equivalent, packed in a two-dimensional hexagonal lattice[15,22]. Concerning the cubic Q phase, six different structures with cubic symmetry have thus far been identified, and their structures have been determined unambiguously[6,24] by using a recently proposed pattern recognition approach[5]. We adopt here the nomenclature introduced by Luzzati's group: a cubic phase is called Q^n, where Q stands for cubic and n is the number of the relative space group, according to the International Tables of Crystallography.

The structure of the Q^{230}, Q^{224} and Q^{229} phases, reported in Fig. 10, can each be described in terms of two three-dimensional networks of joined rods, mutually intertwined and unconnected the rods are respectively linked coplanarly three by three, tetrahedrally four by four and cubically six by six. As originally pointed out by Luzzati and coworkers[6], these three structures are bicontinuous (i.e. both the water and the hydrocarbon media are continuous throughout the structure) and can be visualised as 3-D topological generalizations of the lipid bilayer. The structure of the Q^{212} phase is related to that of Q^{230} one of the two networks of rods is preserved, while the other is replaced by a lattice of closed micelles. Also, this phase consists of two continuous disjoined media, one apolar, containing the micelles, and the other polar the Q^{212} can be visualised as a 3D generalization of the lipid monolayer.

The structure of the last two cubic phases, the Q^{223} and Q^{227}, have been the object of controversial reports and only very recently seem to have been determined unambiguously[10,28]. The two phases consist of two types of closed and disjoined micelles embedded in a continuous matrix (Fig. 11) these structures are non-bicontinuous. It is interesting to notice that examples of the Q^{230} phase have been observed both of type I or II, depending on the chemical composition of the system. Instead, all the known examples of phases Q^{212}, Q^{224}, Q^{227}, and Q^{229} belong to type II and those of phase Q^{223} to type I. Concerning phase behaviour, it must be observed that the most fundamental parameter which controls polymorphic phase behaviour has been identified in the interfacial curvature[9,10,22,28] (Fig. 12); the polymorphism of lyotropic systems appears as a direct consequence of the different types of interactions (crudely schematized in Fig. 13) existing between the polar head groups (through electrostatic forces) and the paraffinic chains (through van der Walls forces). In fact, in amphiphilic systems, head and chains are coupled in the same molecule and neither of them is able to independently minimise its free energy with respect to its cross-sectional area; a compromise is achieved by introduction of symmetry within the structure[21]. Sadoc and Charvolin[28] have discussed how the frustrations in bilayers lead to polymorphism in lyotropic liquid crystals.

3.2. *Phase Equilibria and Phase Diagrams*

For lipid-containing systems, phase transitions may be induced either by varying the temperature or the water concentration[6,15,22]. It is interesting to note at once that for some systems, such as ionic amphiphiles with only one hydrophobic chain, variation of concentration is the more important driving force for inducing transitions between mesophases, so that phase boundaries are close to vertical (the corresponding generalised phase diagram is reported in Fig. 14[22]). On the contrary, when the amphiphile is non-ionic or zwitterionic, and its molecule presents two aliphatic chains, temperature appears to be the most important variable, and many of the phase boundaries appear closer to horizontal than to vertical. In this case, an increase in the temperature plays a role qualitatively similar to that of a decrease in the water content[22].

Concerning the phase diagram reported in Fig. 14, it must be observed that this phase sequence is in agreement with most binary systems studied to date, although as a general rule a given system will not form all of the phases shown[22,29]. As indicated above, the case where phase transitions are driven principally by changes in water content is found in many charged lipid systems, such as alkali carboxylic soaps. In reality, a certain temperature dependence is always observed, so that the phase boundaries can deviate from vertical. Noteworthy is the case of lysophospholipids, which display a similar behaviour even when they are uncharged[30]: as an example, the phase diagram of lauroyl lysophosphatidyl choline is reported in Fig. 15. In general, phase diagrams of such a kind are observed when the lipid presents only a single chain and is considerably less hydrophobic than the corresponding diacyl compounds[22]. In fact, the case of transitions driven predominantly by temperature is more typical of some diacyl zwitterionic phospholipids.

Some examples extracted from Ref. 22 (where the reader will find the original references) are presented in Fig. 15. The phase diagram for saturated C_{20} diacyl phosphatidyl ethanolamine indicates that the Lα phase is only stable over a short range of temperature, while the H phase occurs at high temperatures at all water concentrations out to the excess water region. If, in the lipid polar head, we exchange the ammonium group for a trimethyl ammonium group there is a drastic effect on the phase diagram: as an example, we consider the C_{14} saturated diacyl phosphatydil choline/water system. In this case, as observed for most other chain lengths, only a fluid lamellar Lα phase is seen when the chains are melted, except at very low water contents.

Variation of the length of the aliphatic chains can also have profound effects on phase diagrams. For example, in the case of phosphatidyl ethanolamines with short chains, additional phases appear between the lamellar and the inverse hexagonal phase, as Fig. 15 shows for C_{12} dialkyl phosphatidyl ethanolamine. It is very interesting that these phases include a number of bicontinuous cubic phases Q^{230}, Q^{224} and Q^{229}, but other unidentified nonlamellar phases probably also occur. Referring again to the generalised phase diagram reported in Fig. 14, we recall that a great deal of effort has been spent in recent years in trying to understand even the qualitative features of such phase sequences (reviewed in Ref. 22. See also Ref. 29). In part, this work has been hampered by the difficulty of establishing the structure of the intermediate phases, but perhaps the major problem lies in identifying and quantifying the important molecular interactions which are responsible for mesophase stability. In particular, the many intermediate phases which can be found in locations "a", "b", "c" and "d" of the phase diagrams are only poorly understood. The majority of these phases detected to date are cubic: the ones observed in regions "c" and "d" are of type I, whereas the ones in regions "a" and "b" are the inverse, namely type II. Furthermore, the cubic phases in region "d" are based on an ordered packing of anisotropic micellar aggregates, whereas the phases detected in regions "b" and "c" present structures based upon bicontinuous interfacial headgroup regions.

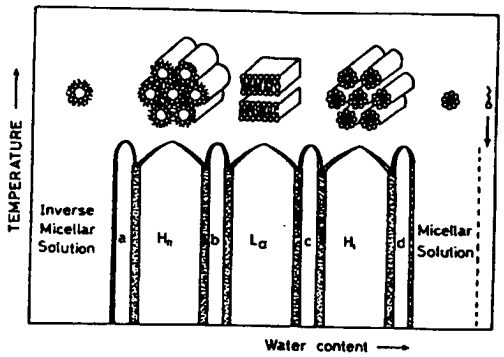

Fig.14. Generalised lyotropic binary phase diagram of lipid-containing systems where phase transitions are mainly determined by changes in water concentration. (From Ref. 22).

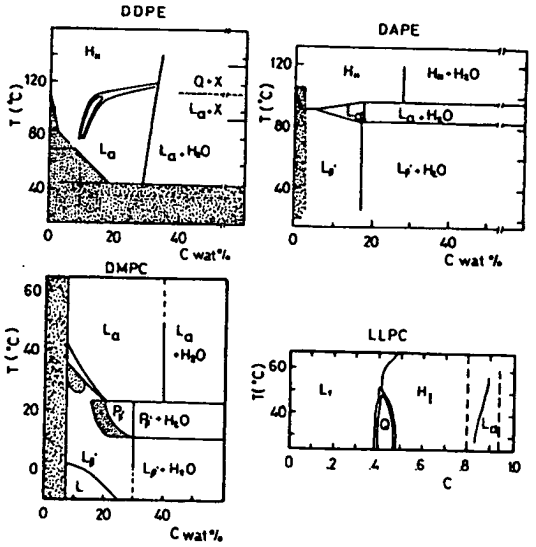

Fig.15. Examples of lyotropic binary phase diagrams. DDPE, didodecyl - phosphatidyl - ethanolamine; DMPC, dimyristoyl - phosphatidyl - choline; DAPE, diarachinoyl - phosphatidyl - ethanolamine; LLPC, lauroyl - lysophosphatidyl - choline. The dotted lines and shaded areas indicate either insufficiently analysed or biphasic regions. C is the lipid weight concentration. (From Ref.s 22 and 30).